NJB, Volume 32(1), June, 2019

INCIDENCE OF SEEDLING BLIGHT DISEASE OF RAPHIA PALM (*RAPHIA HOOKERI* MANN AND WENDL.), THE DISEASE AGENT AND ITS PHYTOTOXIC EFFECT IN THE SEEDLINGS AND NUTRIENT PROPERTY OF SOIL AT THE NIFOR SUB-STATION, ONUEBUM, BAYELSA STATE

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Received 27th February, 2018; accepted 10th December, 2018

ABSTRACT

A study of the incidence of seedling blight disease among *Raphia hookeri* palm seedlings in the Nigerian Institute for Oil Palm Research, *Raphia* Substation, Onuebum, Bayelsa State, Nigeria was carried out to determine the causal agent of the disease, phytotoxic- effect of the causal agent on the seedlings and assess the nutrient status of the *R. hookeri* seedling blocks. The disease incidence was 5%. Advanced stage of seedling blight was characterized by the lamina of the leaflets becoming fragile and gradually falling off, leaving tattered leaflets. Overall soil fertility was adequate, although the soil was poorly drained. Pathogenicity test and molecular analysis revealed that *Diaporthe* sp. was responsible for the disease. Results also showed that *Diaporthe* sp. likely released toxic substances which damaged the photosynthetic layer of the leaf tissues. This is the first report of *Diaporthe* sp. causing seedling blight in *R. hookeri*. Symptoms of the disease were similar to those caused by *Glomerella cingulata* usually seen in the rainy season. A study of the incidence of *G. cingulata* and *Diaporthe* sp in the seedling blight of *R. hookeri* during both rainy and dry seasons is suggested. Improved soil fertility might contribute towards the successful management of seedling blight disease caused by *Diaporthe* sp.

Key words: Incidence, phytotoxic effect, *Raphia* palm, seedling blight, soil property

INTRODUCTION

Raphia hookeri (Mann and Wendl.) is among the eight species in the genus *Raphia* which are indigenous to Nigeria (Otedoh, 1982). Economic uses of *Raphia* include making of ropes, baskets and thatch for roofing as well as palm wine and alcohol for local consumption and industry. Moreover, the fibre from the trunk and leaves has been recommended for paper production (Otedoh, 1975). The cultivation of *Raphia* palm in Nigerian Institute for Oil Palm Research (NIFOR) involves growing it in nurseries before transplanting in the field.

Different pathogens such as *Glomerella cingulata* (Oruade-Dimaro and Ekundayo, 1992), *Xylaria feejeensis* (Esiegbuya *et al*., 2013a) and *Chalara paradoxa* (Esiegbuya *et al*., 2013b) have been isolated from *R. hookeri*. Changes in environmental conditions have been documented to affect the incidence and severity of plant disease and influence the further co-evolution of plants and their pathogens (Chakraborty and Newton, 2011; Eastburn *et al*., 2011).

The symptoms of *G. cingulata* on *R. hookeri* seedlings according to Oruade-Dimaro (1989) began with the youngest fully expanded leaves as transparent yellow circular spots of about 1.0 mm in diameter, appearing on the tips, edges and middle region of leaflets. These circular spots become necrotic**,** surrounded by yellow haloes, and giving an entire spot size of about 2-5 mm in diameter. Such numerous spots coalesced within two weeks from

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The onset of infection, spreading from tip to the base of leaflet (Oruade-Dimaro, 1989). At an advanced stage of the disease, the lamina of the leaflets become fragile and gradually fall off, leaving tattered leaflets (Oruade-Dimaro (1989). Symptoms of *G. cingulata* on *R. hookeri* seedlings are usually observed during the rainy season.

The annual percentage occurrence of seedling blight in NIFOR ranges from 25% to 63% in a single seedling block and usually results in crop loss and reduction in farmer's income (unpublished data). With this background knowledge of the devastating effect of the disease (usually during the rainy season) and the causal agent, it was pertinent to investigate if the same agent was responsible for the observed symptoms in the dry season. This study was, therefore, designed to determine the pathogen causing the symptoms of seedling blight during the dry season, its phytotoxic effect on the seedlings and to evaluate the soil properties of the planting area in order to ascertain the presence of any correlation between disease incidence among *R. hookeri* seedlings and soil fertility.

MATERIALS AND METHODS

Description of study area

The Nigerian Institute for Oil Palm Research (NIFOR) *Raphia* substation is located 2 km off Onuebum –Otuoke Road in Ogbia Local Government Area of Bayelsa State and bounded on one side by Atubu Creek. The substation has 17.5 hectares of *Raphia* palm.

Assessment of the incidence of seedling blight on the R. hookeri seedling blocks

This was done from the months of November 2015 to February 2016, according to the method described by Tarr (1972), by counting the total number of infected plants against the total number of seedlings in a single seedling block. The percentage disease incidence was then determined using the formula:

$$\text{Disease incidence (I)} = \frac{\text{Number of infected plants}}{\text{Total number of seedlings examined}} \times 100$$

Soil sampling and analysis

For the purpose of this assessment, soils were sampled at predetermined depths of 0 – 20 cm (surface) and 20 – 40 cm (subsurface), respectively. This was done using soil auger and the soil samples were transferred into well labeled polyethylene bags. Core samples were also obtained for bulk density and saturated hydraulic conductivity determination. All sampled points were geo-referenced using the hand-held Garmin Global Positioning Systems (GPS) Equipment. A total of 8 soil samples and 4 core samples were collected, processed and analyzed. The soil samples collected were air-dried at room temperature and sieved through a 2 mm sieve .The resulting soil samples were analyzed for their physical and chemical properties as follows: particle size was determined by hydrometer method (Gee and Baulder, 1986); available phosphorus, P (Anderson and Ingram, 1993) and total nitrogen (N) according to Brookes *et al*. (1985). Soil pH was determined in a 1:2 soil to water suspension using a pH meter (Maclean, 1982). The exchangeable bases were extracted with ammonium acetate (NH_4OAC) buffered at pH 7.0

(Thomas, 1982), while potassium (K) and sodium (Na) contents were read from a flame photometer.The exchangeable calcium (Ca) and magnesium (Mg) were determined using atomic absorption spectrophotometer (AOAC, 1990).Total exchangeable acidity ($H^+ + Al^{3+}$) was determined using the method of Anderson and Ingram (1993) and effective cation exchange capacity.

Saturated hydraulic conductivity (K_{sat}) of the soil (its ability to conduct water when all pores are full of water) was determined by direct application of Darcy's Law. A saturated soil column of uniform cross-sectional area and a diameter was subjected to a hydraulic gradient (Topp and Dane, 2002). The resulting flux of water was measured and the value of K_{sat} was calculated using the following equation and method described by Topp and Dane (2002).

$$K_{sat} = \frac{VL}{At\ \Delta H}$$

Where,

V = Volume of water flowing through a cross sectional area
L = Length of column
A = Cross-sectional area of flow through the soil column
t = Time of flow
ΔH = Hydraulic head difference

Isolation and identification of fungi

Portions of *R. hookeri* seedling leaves showing symptoms of seedling blight disease were excised, surface-sterilized by washing samples with sodium hypochlorite solution and then rinsed three times in sterile distilled water, blotted dry with sterile tissue paper, and plated on PDA. The cultures were incubated at room temperature (28 ± 2^oC).

Fungi associated with the seedling blight disease were then sub-cultured on freshly prepared PDA plates and incubated at room temperature for seven days. The cultures were examined for cultural and microscopic characteristics which were used for identification of the isolates according to the methods described by Barnett and Hunter (1998). Isolates identified as described above were sent to the Commonwealth Mycological Institute, Surrey, for confirmation.

In vitro determination of phytotoxin production by the fu**ngal isolates**

The fungal isolates were grown on a basal medium (Czapek-dox broth, CDB) consisting of glucose, 30.0; $NaNo_{3,}$ 2.0; KH_2PO_4, 1.0; $MgSO_{3,}$ 0.5; KCl, 0.5 and $FeSO_{4,}$ 0.01 (g/l distilled water). It was dispensed into 500 ml conical flasks and sterilized in an autoclave at 121^oC for 15 minutes, after which each flask content was inoculated with the identified isolates while Czapek-dox broth medium without the identified fungal isolates served as the control.

Pathogenicity test

This was done following the method described by Odigie *et al*. (2013) by surface-sterilizing the leaves of *R. hookeri* seedlings with 95% ethanol (analytical alcohol, so as to kill any pathogens on the leaf surface) using cotton wool. Thereafter, the leaves were inoculated with a month-old liquid Czapek-dox broth (culture broth for phytotoxic test is usually left for at least one month) containing the phytotoxic substances of each fungal isolate and having spore count of $1.6 - 6.5\times10^5$ per ml (determined by using a haemocytometer). The inoculation was done by puncturing the

Leaves of the *R. hookeri* seedling to expose the tissues for rapid fungal invasion and then spraying 10 ml of inoculum suspension of 1.6 - 6.5×10^5 spores/ml onto the punctured leaves. The seedlings were then covered with sterile Polyethylene bags for three days to encourage a humid environment for the spores of the fungi**.** Un-inoculated seedlings served as control. The treatment and control were then kept in a green house.

After one month of incubation, leaf tissues showing symptoms similar to seedling blight were further evaluated by re-isolating fungi from the inoculated seedlings. The re-isolated fungi from symptomatic inoculated seedlings were compared with the isolates used for the initial inoculation in order to ascertain which of the isolates was the causal agent of the disease.

Effect of phytotoxic substance produced by disease-causal agent on R. hookeri seedlings

This was done by preparing thin sections of the experiment and control leaf samples using a hand-held microtome followed by flooding sections of the leaf samples with 0.1% toluidine blue O (TBO). Excess stain was removed by flooding with distilled water before examining under a microscope fitted with a motic camera.

RESULTS

Out of a total of 400 *R. hookeri* seedlings examined, 20 showed symptoms of seedling blight, representing 5% incidence (Figure 1). The infection spread from tip to the base of leaflet (Figure 2). The symptoms began as transparent yellow circular spots of about 1.0 mm in diameter, appearing on the tips, edges and middle region of leaflets on the youngest fully expanded leaves; these circular spots became necrotic, surrounded by yellow haloes, giving an entire spot size of about 2-5 mm in diameter. The various spots merged within two weeks after infection. At an advanced stage of the disease, the lamina of the leaflets became fragile and gradually fell off, leaving tattered leaflets. In heavy disease attacks, complete death of seedlings occurred while surviving seedlings were characterized by thrifty growth.

Phytotoxic effect of causal agent of disease on *R. hookeri* seedling tissues showed partial loss of green pigmentation by the leaves indicated by reddish purple lesions (Figure 3) while latter stages of the disease showed a completely damaged photosynthetic layer (Figure 4, arrowed). The result of the control experiment did not produce any symptoms on the plant tissues.

The fungus indicted in the pathogenicity test as being the cause of the disease was of greyish white coloration when grown on PDA (Figure 5) and was fluffy while on the reverse side of culture; it was whitish to beige colour which later turned dark brown. The fungus produced filiform spores on PDA after 3-7 days of growth. The results of molecular identification of this fungal isolate using the ITS sequence obtained from the isolate, produced top matches at >99% identity to multiple sequences from members of the genus *Diaporthe* and their anamorphic state. The phytotoxic substance produced by *Diaporthe* sp. caused symptoms similar to those observed in the field.

The soil colour ranged from very dark greyish brown 10YR 3/2 to light yellowish brown 10YR 5/6 (Table 1).The soil was sandy loam (SL) to loamy sand (LS) both at the surface and subsurface levels. Also, the soil was poorly drained as shown by the low value of saturated hydraulic conductivity (ksat) of 0.001 to 0.00 cm^3/hr, abundant mottles (A, A) in the subsurface horizon and a colour hue of 10YR in both surface and subsurface horizons (Soil Survey Manual, 1993).The soil was acidic with a pH of 4.8 to 5.1. The percentage organic carbon contents were low to moderate (0.42 to 1.95%).The total nitrogen content was considerably low, with the values ranging from 0.027 to 0.132%; this is considered low compared with the standard rating. Similarly, available phosphorus content was low and ranged from 2.64 to 12.8 mg/kg (FFD and NPFS, 2011). The effective cation exchange capacity (ECEC) of the soils was low to moderate (5.599.71-9.71 cmol/kg). The values of magnesium (Mg) and potassium

(K) were high in the entire study area (Table 2) compared with the standard rating (FFD and NPFS, 2011). Therefore, the overall fertility of the soil was moderate.

DISCUSSION

In this study, *Diaporthe* sp. showing symptoms similar to those of *G. cingulata* was isolated from the *R. hookeri* seedlings and pathogenicity test implicated *Diaporthe* sp as the cause of the disease.

Different pathogens such as *Glomerella cingulata* (Oruade-Dimaro and Ekundayo, 1992), *Xylaria feejeensis* (Esiegbuya *et al*., 2013a) and *Chalara paradoxa* (Esiegbuya *et al*., 2013b) have been isolated from *R. hookeri*. A significant percentage of this disease reported earlier might have been due to *Diaporthe* sp. but mistaken for *G. cingulata* in previous reports. Whereas *G. cingulata* was reported to have caused disease in *R. hookeri* during the wet season, according to Oruade-Dimaro and Ekundayo (1992). *Diaporthe* sp. was isolated during the dry season (November to February) in the current study. Caruso and Ramsdell (1995) reported that the genus *Diaporthe* caused a number of diseases such as twig blight, fruit rot and canker of blueberry, upright dieback and viscid rot of cranberry but this is the first report of *Diaporthe* to be implicated in any disease of *R. hookeri*. The pathogen has also been reported to be associated with weeds and this makes weeds a potential source of *Diaporthe* inoculum for cultivated plants (Roy *et al*., 1997; Mengistu and Reddy, 2005; Vrandecic *et al*., 2006). However, in this study, weeds around the seedling blocks were not examined for the presence of *Diaporthe*. The diversity of pathogens isolated from *R. hookeri* palm seedlings might be attributed to environmental factors or host specificity.

According to Commonwealth identification report on this study and other reports (Gomes *et al*., 2013; Lawrence *et al*., 2015), members of the genus *Diaporthe* and their *Phomopsis* anamorphs are plant pathogens of a wide range of hosts, including economically important crops. They are also found as saprobes or endophytes.

The interaction between the *Diaporthe* sp. and the host tissue gave rise to the damage (chlorosis and disintegration) of its photosynthetic layers which confirmed the invasion of the host by the pathogen. However, the type of metabolic substance produced by the fungus was not determined in this study. Metabolites of *Diaporthe* sp. have been reported to possess herbicidal activity (Maiquel *et al*., 2016). According to the authors, the metabolites of the pathogen have a broad-spectrum action in both dicotyledonous (e.g. *Glycine max*) and monocotyledonous (e.g. *Oryza sativa*) seeds. Souza *et al*. (2015) also obtained an efficiency of 100% in the pre-emergence of *Cumumis sativus* (dicotyledonous) and *Sorghum* sp. (monocotyledonous) seeds when inoculated with fermented broth of *Diaporthe* sp. This corroborates the herbicidal nature of *Diaporthe* sp metabolites. Further investigation is required in this regard as the composition of the metabolites has to be studied. Maiquel *et al*. (2016) stated that the secondary metabolites of *Diaporthe* sp. may be related to molecules from the terpenoid groups which are produced by a wide range of fungi. Terpenoids are reported to have hormonal functions and inhibit plant growth (Castro *et al*., 2001). This property maybe the mechanism by which *Diaporthe* sp. causes blight in *R. hookeri* seedlings.

Considering the soil properties, the overall soil fertility was moderate according to conventional rating (USDA, 1993). Good soil fertility reduces incidence and severity of plant diseases. A follow- up research would be to determine the effect of alteration of the individual soil factors with a view to improving soil fertility.

In this study, nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg) sodium (Na) and potassium (K) were detected in varied amounts in the soil samples. Nutrients such as Ca suppress diseases by increasing the structural integrity and resistance of the middle lamella, cell wall components, and cell membranes to the extracellular macerating enzymes produced by pathogens (Bateman and Basham, 1976; Kelman *et al*., 1989).

Nitrate sources such as N suppress production of enzymes produced by pathogen, while ammonium may increase them (Huber and Graham, 1999). Magnesium is also important for structural integrity of cell components and also reduces susceptibility to pathogen produced by macerating enzymes as long as Ca levels remain sufficient (Csinos and Bell, 1989). Silicon is combined with other components to give cell walls greater strength as physical barriers to penetration and is involved in physiological responses to infection by increasing the availability of K and mobility of Mn (Savant *et al*., 1997). The ability of the *R. hookeri* seedlings to thrive in the prevalence of *Diaporthe* sp. might be attributed to these soil nutrients.

Soil properties consist of both micro- and macro- elements. The direct effect of micro- and macro- elements includes production of defense-related compounds, while the indirect effects include soil nitrification, pH and chemical transformation of micronutrients (Elmer, 2015). Soil nutrient sufficiency has been reported to provide a general form of disease resistance by maintaining a high level of inhibitory compounds in tissue or a quick response to invasion by a pathogen (Huber and Graham, 1999). There are pathogens that can immobilize nutrients in the rhizophere or in infected tissues such as roots, while others interfere with translocation or utilization efficiency and can cause nutrient deficiency or hyper-accumulation and nutrient toxicity (Huber and Graham, 1999).

Diaporthe sp. was responsible for blight disease of *R. hookeri* seedlings during the period of the study. Certain metabolites produced by *Diaporthe* sp. caused chlorosis and disintegration in the photosynthetic layer of the leaf tissues of *R. hookeri* seedlings. Care should be taken in the process of identification of seedling blight pathogens of *R. hookeri* so as to avoid mis-identification. This is the first report of *Diaporthe* sp. being isolated from and causing blight in *R. hookeri* seedlings. Improved soil fertility might contribute towards the successful management of seedling blight caused by *Diaporthe* species in *R. hookeri*.

ACKNOWLEDGEMENT

This work was funded by the Tertiary Education Trust Fund (TETFund) grant, for which authors are grateful.

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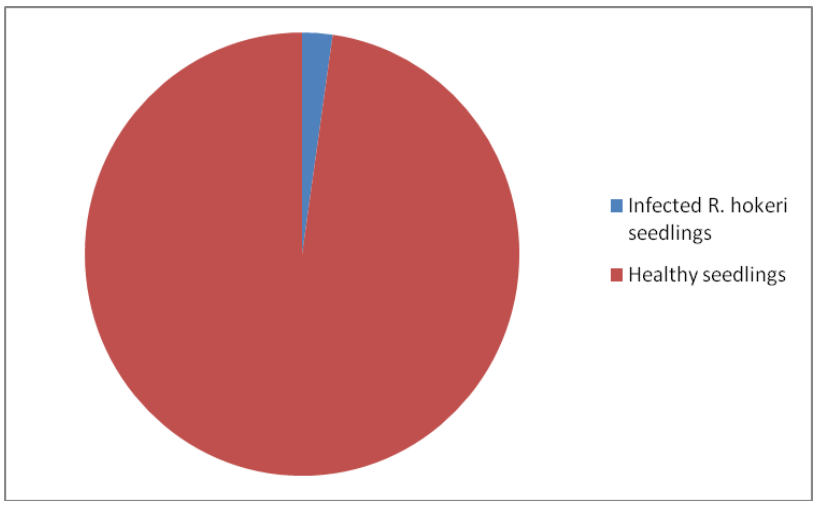

Figure 1: Incidence of seedling blight caused by *Diaporthe* sp. in a *R. hookeri* plantation.

Figure 2: Symptoms of seedling blight disease in *R. hookeri* seedlings

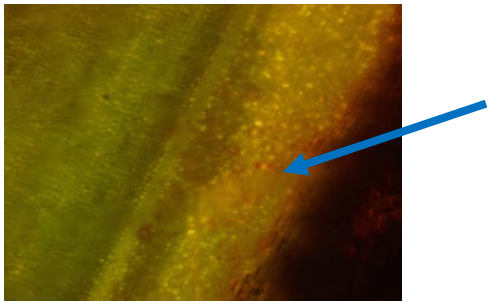

Figure 3: Leaf lamina of *Raphia hookeri* showing lesions and onset of chlorosis (arrowed) caused by *Diaporthe* sp.

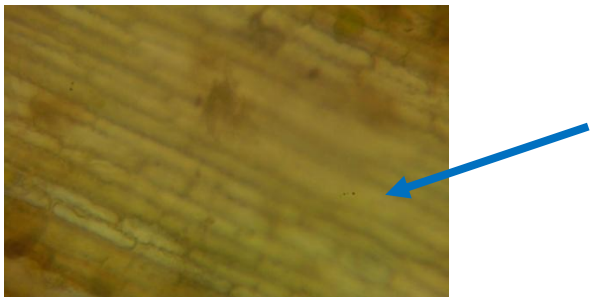

Figure 4: Longitudinal section of *R.hookeri* leaf tissue showing chlorotic and disintegrated photosynthetic layer (arrowed) caused by *Diaporthe* sp.

Figure 5 : A four- day old culture of *Diaporthe* sp. Grown on PDA..

Table 1: Morphological and physical properties of the soil at R. hookeri Substation, Onuebum, Bayelsa State

Sample ID	Depth (cm)	Colour	Mottle	Structure	Ksat (hr/cm)	Clay g/kg	Silt g/kg	Sand g/kg	Textural class
UN1	0 -20	10YR3/2 very dark greyish brown	N	GR	0.001	38	152	810	LS
UN2	20 – 40	10YR 4/3 Brown	F	SAB	0.00	78	132	790	SL
UN3	0 – 20	10YR 5/4 Yellowish brown	N	SAB	0.01	48	188	765	SL
UN4	20 – 40	10YR 5/6 Yellowish brown	A,A	SAB	0.001	103	142	755	SL
UN5	0 – 20	10YR6/4 Light yellowish brown	N	SAB	0.001	63	167	770	SL
UN6	20 – 40	10YR 6/6 Brownish yellow	A,A	SAB	0.001	123	147	730	SL
UN7	0 – 20	10YR 4/2 Dark greyish brown	F	CR	0.00	53	147	800	LS
UN8	20 – 40	10YR 5/2 Greyish brown	A,A	SAB	0.00	73	127	800	LS

Mottles: N = None, F, M = Few and medium, C = Common, A, A = Abundant and Coarse.
Structure: GR = granular, SA B= Sub-angular blocky,
Textural class: SL = Sandy loam, LS = Loamy sand
Ksat = Saturated hydraulic conductivit

Table 2: Chemical properties of the soil at *R. hookeri* Substation, Onuebum, Bayelsa State

Sample ID	Depth (cm)	pH	OC	N	P	Ca	Mg	Na	K	H	Al	ECEC
				%	Mg/kg				Cmol/kg			
UN1	0 -20	5.1	1.95	0.132	9.09	3.60	2.48	0.38	2.05	0.80	0.40	9.71
UN2	20 – 40	4.9	1.28	0.089	5.11	2.80	0.80	0.23	0.13	1.00	1.50	6.46
UN3	0 – 20	5.0	1.44	0.120	5.16	2.24	0.48	0.21	1.46	0.60	0.60	5.59
UN4	20 – 40	4.8	0.80	0.057	3.65	2.24	0.32	0.20	0.11	0.80	0.70	4.37
UN5	0 – 20	4.9	1.02	0.081	6.20	2.08	1.12	0.25	2.52	0.80	1.10	8.67
UN6	20 – 40	4.8	0.42	0.027	2.64	2.00	0.56	0.16	1.68	0.80	1.60	6.8
UN7	0 – 20	5.0	1.76	0.127	12.84	3.76	0.80	0.32	1.49	0.90	0.60	7.87
UN8	20 – 40	4.8	1.09	0.073	7.56	3.20	0.40	0.29	0.98	0.70	1.00	6.57

OC: Organic compound.
ECEC: Effective cation exchange capacity.