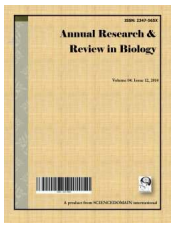

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Characteristic Variations in *Lasiodiplodia theobromae*; Pathogen of inflorescence Dieback of Cashew in Growing Ecologies of Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author DOA designed the study, wrote the protocol, wrote the initial manuscript and anchored the laboratory study, also gathered the initial data and performed preliminary data analysis. Author DBO finalize the manuscript and performed the statistical analysis, while author AJ managed the literature searches and the analyses of the data. All authors read and approved the final manuscript.

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ABSTRACT

Nine isolates of *Lasiodiplodia theobromae* were collected from cashew inflorescences showing typical symptoms of dieback disease in nine different farms belonging to various cashew growing ecologies of Nigeria. The result revealed that most of the *L. theobromae* isolates exhibited significant differences in morphology, colour and spore dimensions. The colony growth rate of *L. theobromae* range from 11.95 mm to 14.17 mm, colony texture and colour of the isolates in the obverse were fluffy dark mouse grey, fluffy mouse grey, fluffy olivaceous grey or fluffy groh grey while the reverse colour of the isolates was either greyish blue or sky grey. Sporulation was

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observed at varied degrees in all the *L. theobromae* isolates except in isolates from Oro and Ejule and likewise is the numbers of pycnidia produced varied in all the isolates across growing ecologies. Significant variations were observed in the characters and morphology of the *L. theobromae* isolates causing inflorescence dieback of cashew in Nigeria.

Keywords: Cashew; morphology; growth; pigmentation; sporulation; pycnidia.

1. INTRODUCTION

Lasiodiplodia species are common, especially in tropical and subtropical regions where they cause a variety of diseases [1], according to [2] the genus is based on *Lasiodiplodia theobromae*. The main features that distinguish this genus from other closely related genera are the presence of pycnidial paraphyses and longitudinal striations on mature conidia. So far, 20 species have been described and are differentiated on the basis of conidial and paraphyses morphology. The more recently described species have been separated not only on morphology, but also on the basis of sequence data, and such studies have confirmed and new species have been described since 2004 [3-7] There have been very few studies on the characterization of *Lasiodiplodia* species in Nigeria apart from few reports on the control of *L. theobromae* and its association with cashew disease complex [8-11]. In a survey of inflorescence dieback of cashew across agro-ecologies of Nigeria some variations were observed in the cultures of *Lasiodiplodia* isolates collected on cashew. Even if the epidemiology of the disease caused by these fungi in cashew is at present poorly understood, their occurrence shows a regional variability, apparently linked also to climatic differences. Among other species *Lasiodiplodia theobromae* is reported (usually as the teleomorph stage "*Botryosphaeria" rhodina*) as a tropical and sub-tropical pathogen on many different tree species [12]. It is frequently isolated from cashew inflorescent dieback across cashew growing ecologies of Nigeria. The objective of the study is to characterize *Lasiodiplodia theobromae* isolates to establish their biodiversity in cashew agro-ecologies of Nigeria.

2. MATERIALS AND METHODS

2.1 Source of *Lasiodiplodia theobromae* Isolates

Isolates of *Lasiodiplodia theobromae* were cultured from inflorescences showing typical symptoms of dieback disease of cashew in major cashew growing ecologies of Nigeria. The isolates; L-Och_2, L-Oro_3, L-Otp_4, L-Odp_4, L-Ejl_2, L-Gam_3, L-Oba_5, L-Ogb_1 and Lbd_1 were collected from growing locations; Ochaja, Oro, Otukpa, Odoba - Otukpa, Ejule, Ganmo, Obollo - Afor, Ogbomoso and Ibadan respectively. The isolates L-Och_2, L-Oro_3, L-Otp_4, L-Odp_4, L-Ejl_2, and L-Gam_3 were from North Central, L-Ogb_1 and Lbd_1 from South Western and L-Oba_5 from South Eastern, Nigeria. Each isolate was maintained in pure culture on PDA, the stock culture of each isolate was revived by cutting the edge of mycelia mat using sterile cork borer, placing on Potato Dextrose Agar (Lab M Limited, Heywood, Lancashire, UK) and the petri dishes were incubated for 5-7 days at 28±2℃.

2.2 Growth Rate of *Lasiodiplodia theobromae* Isolates

Isolates of *L. theobromae* were cultured on Potato Dextrose Agar (PDA) on petri dish (85 mm) for comparison of morphological and growth characters. Five millimeter (5 mm) discs from margins of actively growing 7-day-old culture of each isolates were inoculated at the center of petri dishes containing half strength PDA and replicated three times. The inoculated plates were incubated at 28±2℃. Colony growth rate was recorded by measuring colony diameter after 24, 48, 72, 96, 120, and 144 hours of incubation.

2.3 Color Pigmentation of *Lasiodiplodia theobromae* Isolates

Isolates cultured and incubated as stated above. Colony colour and texture observations were made with the aid of a colour chart by Rayner [13] and recorded after 7 days of incubation.

2.4 Sporulation and Pcynidia Production in *Lasiodiplodia theobromae* Isolates

With the aid of microscope, observation of day of sporulation of the isolates began from second day of inoculation and recorded per isolates. Isolates of *L. theobromae* were cultured on water agar and incubated for 15 days; the grown culture was harvested by flooding the petri dish and washing the culture through Whatman No 1

filter paper. The residue on the filter paper was left to dry for 5 minutes and the pcynidia were visually counted.

2.5 Conidia Dimension and Septation of *Lasiodiplodia theobromae* Isolates

L. theobromae isolates were grown on half strength PDA for 14 – 20 days depending on day of sporulation. The pcynidia were harvested and with the aid of a sterile inoculating needle transferred onto a clean slide and gently tease with drops of lactophenol cotton blue on slide to exude the conidia and a cover slip was gently over laid. With the aid of a microscope at x25 objective, about 30 mature conidia were measure for each *L. theobromae* isolates to determine the length and breadth of conidia as well as length and breadth of septation.

3. RESULTS AND DISCUSSION

The result of morphological characters of *L. theobromae* revealed that the fastest growing isolate of *L. theobromae* (L-Ogb_1) is from Ogbomoso, this covered the entire petridish in 96 hours. L-Ogb_1 recorded the mean mycelial growth of 68.21 mm but not significantly different from Otukpa isolate (L-Otp_4) with 67.75 mm and Ejule isolate (L-Ejl_2) showing 65.75 mm growth. The Ochaja isolate showed the least mean mycelia growth of 47.72 mm but not significantly different from Oro (49.49 mm). The mean mycelia growth of *L. theobromae* isolates from Ganmo (58.94 mm), Ibadan (58.14 mm), Obbolo-Afor (56.88 mm) and Odoba-Otukpa (55.63 mm) were not significantly different to one another by Duncan Multiple Range Test. However, they differ significantly from isolates from other study location (Table 1).

The isolates of *L. theobromae* from cashew agro-ecologies of Nigeria have mycelia extension at varied degree for 144 hours on half strength PDA. The colony growth rate of *L. theobromae* isolates range from 11.95 mm to 14.17 mm, however not significant difference. The colony texture and colour of the isolates have limited variations, colour of the obverse (upper surface of mycelia mat) can be categorize into fluffy dark mouse grey, fluffy mouse grey, fluffy olivaceous grey and fluffy groh grey while the reverse (lower surface of mycelia mat) colour was either greyish blue or sky grey.

Isolates L-Ibd, L-Ejl and L-Oba have similar colony textures and colour; fluffy dark mouse grey while fluffy olivaceous grey texture was common to L-Gam and L- Ogb isolates and only isolate L-Oro differ with sky blue colony in the reverse but others are greyish blue except L-Odp having fluffy groh grey on the obverse. No sporulation was observed in L-Oro and L-Ejl till 35 days after incubation while others sporulate at varied degrees. L-Otp, L-Gam and L-Ogb sporulate at day 10 after incubation, followed by L-Oba, L-Ibd and L-Odp on day 12 and sporulation was observed day 18 after incubation in L-Och isolate (Table 2). The numbers of pycnidia produced also differ in all the isolates across the locations with isolate from Ganmo having the highest of 260 pycnidia followed by 171 pycnidia in Otukpa and the least pycnidia in Odoba-Otukpa except Oro and Ejule isolates that with no sporulation (Table 2). Typical morphological features of *L. theobromae* with pycnidia, difference of the mature and immature conidia and the conidiogenous cells were as illustrated in Figs. 1 a – d.

Table 1. Colony growth of *Lasiodiplodia theobromae* isolates

***L. theobromae* isolates**	**Mycelia growth (mm)**						**Mean mycelia growth**
	24h	**48h**	**72h**	**96h**	**120h**	**144h**	
L-Ibd_1	*14.75^{bcd}	41.92^{bcd}	60.79^{ab}	70.92^{ab}	78.84^{ab}	81.42^{ab}	58.14^{ab}
L-Och_2	9.75^{de}	31.92^{d}	47.25^{b}	57.58^{b}	67.74^{b}	72.09^{b}	47.72^{b}
L-Oro_3	10.84^{cde}	31.48^{d}	53.34^{b}	61.84^{b}	67.84^{b}	71.67^{b}	49.49^{b}
L-Otp_4	14.09^{cd}	55.67^{ab}	81.75^{a}	85^{a}	85^{a}	85^{a}	67.75^{a}
L-Ejl_2	23.5^{a}	58.92^{a}	70.5^{ab}	74.67^{ab}	82.92^{a}	84.59^{a}	65.75^{a}
L-Gam_3	8.84^{e}	38.5^{cd}	62.5^{ab}	74.92^{ab}	83.84^{a}	85^{a}	58.94^{ab}
L-Oba_5	15^{cbd}	42.34^{bcd}	54.84^{b}	71.25^{ab}	77.84^{ab}	80^{ab}	56.88^{ab}
L-Odp_4	16^{bc}	41.7^{bcd}	53.92^{b}	69.09^{ab}	73.09^{ab}	80^{ab}	55.63^{ab}
L-Ogb_1	20.34^{ab}	53.84^{abc}	80.92^{a}	85^{a}	85^{a}	85^{a}	68.21^{a}

**Means followed by the same letter in each column are not statistically different (P = 0.05) according to DMRT. L = Lasiodiplodia theobromae; Ibd = Ibadan; Och = Ochaja; Oro = Oro; Otp = Otukpa; Ejl = Ejule; Gam = Ganmo; Oba = Obollo-Afor; Odp = Odoba-Otukpa; Ogb = Ogbomosho; 1 = Oyo State; 2 = Kogi State; 3 = Kwara State; 4 = Benue State; 5 = Enugu State*

Table 2. Morphological descriptions of *Lasiodiplodia theobromae* isolates

L. theobromae isolates	Sporulation	Day of sporulation	Number of pycnidia	Colony growth rate (mm/day)	Colony texture and color	
					Obverse	Reverse
#L-Ibd$_1$	+	12	*77^c	13.57^a	Fluffy dark mouse grey	Greyish blue
L-Och$_2$	+	18	34^e	12.02^a	Fluffy mouse grey	Greyish blue
L-Oro$_3$	-	-	0^h	11.95^a	Fluffy mouse grey	Sky grey
L-Otp$_4$	+	10	171^b	14.17^a	Fluffy mouse grey	Greyish blue
L-Ejl$_2$	-	-	0^h	14.09^a	Fluffy dark mouse grey	Greyish blue
L-Gam$_3$	+	10	260^a	14.17^a	Fluffy olivaceous grey	Greyish blue
L-Oba$_5$	+	12	49^d	13.33^a	Fluffy dark mouse grey	Greyish blue
L-Odp$_4$	+	12	11^g	13.33^a	Fluffy groh grey	Greyish blue
L-Ogb$_1$	+	10	12^h	14.17^a	Fluffy olivaceous grey	Greyish blue

**Means followed by the same letter in each column are not statistically different (P = 0.05) according to DMRT. L = Lasiodiplodia theobromae; Ibd = Ibadan; Och = Ochaja; Oro = Oro; Otp = Otukpa; Ejl = Ejule; Gam = Ganmo; Oba = Obollo-Afor; Odp = Odoba-Otukpa; Ogb = Ogbomosho; 1 = Oyo State; 2 = Kogi State; 3 = Kwara State; 4 = Benue State; 5 = Enugu State*

Table 3. Morphological characters of *Lasiodiplodia theobromae* isolates

Isolates	Conidia dimensions		Paraphyses		Septate size
	Conidia size	L/W ratio	Septation	No of septa	
#L-Ibd$_1$	27.3–42.9 x 15.6– 23.4 µm	1.8	Septate	1	11.7– 15.6 x 3.9 – 11.7 µm
L-Och$_2$	35.1–50.7 x 19.5– 23.4 µm	2	Septate	1	11.7 – 19.5 x 3.9 - 11.7 µm
L-Otp$_4$	27.3–46.8 x 19.5 23.4 µm	1.7	Septate	1	11.7– 15.6 x 3.9 - 11.7 µm
L-Gam$_3$	27.3–39 x 19.5 – 23.4 µm	1.5	Septate	1	11.7 – 19.5 x 3.9 – 7.8 µm
L-Oba$_5$	31.2–42.9 x 15.6 – 19.5 µm	2.1	Septate	1	11.7– 15.6 x 3.9 – 11.7 µm
L-Odp$_4$	27.3–35.1 x 15.6 – 19.5 µm	1.8	Septate	1	11.7– 15.6 x 3.9 – 11.7 µm
L-Ogb$_1$	31.2–42.9 x 11.7 – 23.4 µm	2.1	Septate	1	11.7 – 15.6 x 3.9 – 7.8 µm

#*L = Lasiodiplodia theobromae; Ibd = Ibadan; Och = Ochaja; Otp = Otukpa; Gam = Ganmo; Oba = Obollo-Afor; Odp = Odoba-Otukpa; Ogb = Ogbomosho; 1 = Oyo State; 2 = Kogi State; 3 = Kwara State; 4 = Benue State; 5 = Enugu State*

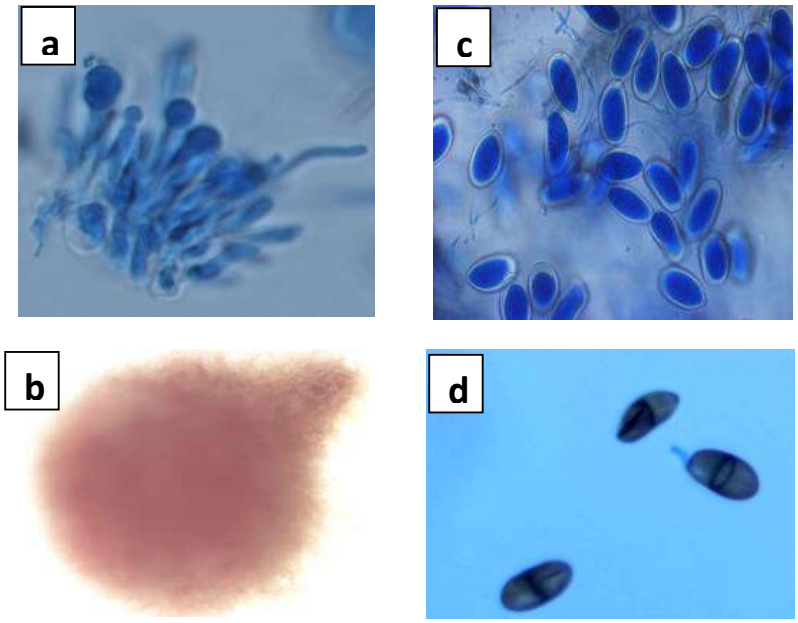

Fig. 1. (a) Conidiogenous cells and young hyaline and thick-walled *L. theobromae* conidia, with non-septate paraphyses indicated by black arrows. (b) Pycnidia. (c) Immature conidia. (d) Mature conidia showing striation and septation

Another important morphological characteristic for the identification of *L. theobromae* was the presence of aseptate paraphyses observed in immature pycnidia. This feature was reported in a recent study, in which aseptate paraphyses along with conidial size were the primary morphological characteristics to separate *L. theobromae* from other *Lasiodiplodia* spp. found in the tropics [5].

The conidia were similar to those described for *L. theobromae* in shape, colour and striation as reported by Punithalingam [14]. Table 3 shows levels of variations observed in the conidia dimension of isolates of *L. theobromae* from study locations. All the isolates had one septa but the septate size of 11.7 – 15.6 x 3.9 – 11.7 µm was common to isolates from Ibadan, Otukpa, Obollo-Afor and Odoba-Otukpa and septate of other isolates varied in size. The conidia length to width ratio of 2.1 was common to Ogbomoso and Obollo-Afor isolates, 1.8 ratio recorded in Ibadan and Odoba-Otukpa isolates, while other isolates had varied ratio. The conidia size of all isolates varied but range from 27.3 – 35.1 x 15.6 – 19.5 µm in Odoba-Otukpa to 35.1 – 50.7 x 19.5 – 23.4 µm recorded in Ochaja isolates. Variation in the fungus has been observed Sutton [2] and Alam et al. [15] showed that some isolates of *B. theobromae* produced pigment in PDA medium and aerial mycelium. Ammar [16] Alves et al. [7] and Shah et al. [17] reported high existence of genetic variation among isolates of the fungus.

4. CONCLUSION

Isolates of *Lasiodiplodia theobromae* from inflorescence dieback disease in cashew growing ecologies of Nigeia exhibit variation in morphological characters. These descriptions on cashew disease in Nigeria is novel and the study can be furthered using molecular tools to characterize the isolates of *L. theobromae* which is equally pathogenic on a number of other indigenous and exotic economic crops.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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