



Research Article

Premature Nut Fall Disease of Coconut: A Case Study of *Botryodiplodia Theobromae* and Other Fungi Associated with The Disease

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ABSTRACT

Coconut (*Cocos nucifera* L.) is grown in several countries by millions of farmers globally. It is a staple food and a cash crop in many developing countries. Generally, about one-third of the buttons produced in an inflorescence develop into mature nuts (that is, the number of buttons made in a bunch often exceeds the number of nuts reaching maturity). Coconut production is affected by several factors, including diseases, which play a significant role. Premature nut fall disease is a common problem encountered by coconut growers. The disease decreases the production potential of the coconut palm, causing considerable yield loss. Hence, the study was carried out to investigate the incidence of coconut's premature nut fall disease over two overlapping growing seasons in two major coconut-growing areas. The Green dwarf (GD) coconut variety has a higher PDI of premature-nut-fall in both growing seasons and locations than other coconut varieties used in this study. The study also revealed high variability in the PDI of premature-nut-fall across coconut varieties, sampling period in both growing seasons, and locations. In both locations and growing seasons, the high percentage of fungi isolated from fallen nuts indicates the role of fungi as pathogens in premature-nut-fall disease of coconut. Furthermore, isolation of *B. theobromae* from the internal tissues of diseased nuts and other floral parts of the coconut indicates that the fungus is responsible for the premature-nut-fall disease of the coconut resulting from fungal infection.

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1. INTRODUCTION

The coconut palm (*Cocos nucifera* L.) is an essential economic crop throughout the tropics and subtropics (James, 1983), although its center of origin is not precisely known. Coconut is grown in about 90 countries worldwide, by an estimated 11 million farmers across 12 million hectares (Adkins et al., 2006; FAO, 2014; Gurr et al., 2016). The crop is mainly grown in Southern and Western Nigeria, and the highest concentration is found in Badagry Local Government area of Lagos State along the Atlantic

coastal line (Ojomo et al., 2010). Coconut is a staple food and a cash crop in many developing countries (Bourke & Harwood, 2009). Generally, about one-third of the buttons produced in an inflorescence develop into mature nuts; the number of buttons made in a bunch often exceeds the number of nuts reaching maturity (Rajapakse et al., 1995). Coconut production is affected by several factors, including diseases, which play a significant role. The premature-nut-fall (also called immature and mature nut shedding) disease is a common problem of coconut (Rajapakse et al., 1995). Shedding of immature and mature nuts decreases the production potential of the coconut palm

(Sudhakara, 1991). The process includes the fall of fertilized or unfertilized coconut fruits during any stage prior to maturity (Sudhakara, 1991). Premature nut fall of coconut has been attributed to various factors such as natural capacity for production, defective fertilization, physiological and environmental factors, disease, and pest damage (Rajapakse et al., 1995).

Fungal infection has been identified as one of the leading causes of premature-nut-fall disease of coconut (Quillec et al., 1984). However, further detailed studies were not conducted to validate such claims, necessitating this study. Among other fungal pathogens, *Botryodiplodia theobromae* has been identified as the primary cause of premature nut fall disease of coconut (Venugopal & Chandra Mohanan, 2006). The organism enters the coconut fruit through a crack on the surface of the fruit or the spikelet or from the point of attachment of the fruit to the spikelet and spreads through the endocarp and endosperm, and gradually spreads outward towards the mesocarp, causing severe damage to the fruit. Furthermore, early signs and symptoms of infected fruits appear as light or dark brown lesions turning into whitish-grey with a shriveled appearance. The infected fruits later turn blackish with cracks at the basal part of the fruit, causing the fruit to fall off prematurely from the bunch. Liquid exudates (water oozing) might also appear on the affected fruit (Dheepa et al., 2018). Liquid exudates (water oozing) might also appear on the affected fruit (Dheepa et al., 2018). In the study, emphasis is given to investigate and document cases of the premature-nut-fall disease of coconut concerning *Botryodiplodia theobromae* and other fungi associated with the disease.

2. METHOD

Sampling was done (using simple random sampling) in 4 quarters during the dry and rainy seasons (twenty-four months) in two locations, namely, Nigerian Institute for Oil Palm Research (NIFOR) Main Station and Coconut Garden, Isihor, in Ovia-North-East Local Government Area, Edo State, Nigeria. Geographic coordinates of each location/field were recorded using a hand-held global positioning system (GPS). NIFOR Main Station, Benin City, with latitude (06.33oN) and longitude (05.37oE), while Coconut Garden, Isihor, Benin City, with latitude (06.39oN) and longitude (5.61oE) respectively. Both locations' climate is subtropical humid, and the entire year can be classified into two distinct seasons, viz., (1) dry season, which starts around October and extends up to March, (2) rainy season, which starts around April and extends up to September. However, there is slight variability in both seasons. Source of coconut fruits Freshly fallen nuts found around the base of the coconut palm were

collected (and recorded daily) from coconut plantations cultivated with different coconut varieties, viz.; green dwarf (GD), orange dwarf (OD), yellow dwarf (YD), red dwarf (RD) also called Sri Lanka brown}, and the fallen nuts were documented as premature-nut-fall. Thus, samples consisted of nuts of different stages of maturity, from button to mature nuts.

The coconut samples collected were preserved in transparent screw cap plastic containers for the isolation of the causal agent and other fungi associated with the disease. Isolation and identification of fungi from fruits and floral parts of the coconut. Freshly fallen nuts with or without signs of rot at the distal end of internal tissues were used to isolate fungi (also, the layer of external husk was removed from nuts without any external symptoms to observe internal infection, if any). In addition, fungi were also isolated from the following coconut floral parts: calyx, spikelet, floret, and inflorescence. Coconut tissues and other floral parts were cut into pieces with a sterile knife (Venugopal & Chandra Mohanan, 2006). Fungi were isolated from the coconut tissue (following surface sterilization of the tissues) using the direct plating technique on potato dextrose agar (PDA). In contrast, the pour plate technique isolated fungi from the coconut floral parts mentioned. The plates were incubated at 28±2°C for 3 to 7 days (Phipps & Porter, 1998; Rangaswami et al., 1975). The fungal growths were aseptically subcultured into a fresh PDA, and each fungus was identified based on cultural and morphological characteristics as described by the Commonwealth Mycological Institute (CMI) (Venugopal & Chandra Mohanan, 2006). Statistical analytical data obtained from the coconut fruit showing signs and symptoms of fruit rot were calculated using the disease incidence (PDI) percentage in a Microsoft Excel Worksheet.

3. RESULTS AND DISCUSSION

3.1. Results

Coconut fruit population in the 2021-2023 growing season. During the growing season (Apr 2021 - Mar 2022), a total of 1922 coconut fruits in the coconut field at NIFOR Main Station were examined for premature-nut-fall, while 2238 coconut fruits in the Coconut Garden, Isihor, were examined for premature-nut-fall (Table 1). During the growing season (Apr 2022 - Mar 2023), a total of 2536 coconut fruits in the coconut field at NIFOR Main Station were examined for premature-nut-fall, while 2068 coconut fruits in the Coconut Garden, Isihor, were examined for premature-nut-fall (Table 1). Coconut fruit varietal population in 2021-2023 growing season During the growing season (Apr 2021 - Mar 2022); in NIFOR Main Station, a total of 638 GD, 487 OD, 422 YD and 375 RD

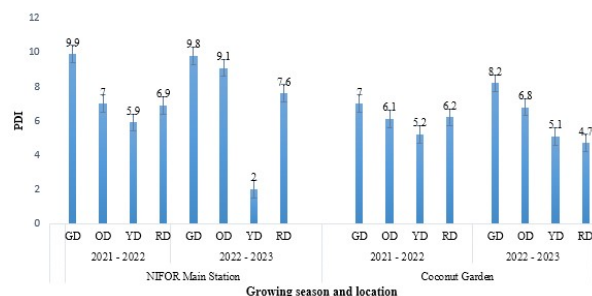
coconut fruits were examined for premature-nut-fall disease, while in Coconut Garden, Isihor, 627 GD, 545 OD, 500 YD and 566 RD coconut fruits were examined for premature-nut-fall disease (Table 2).

During the growing season (Apr 2022 - Mar 2023); in NIFOR Main Station, a total of 921 GD, 561 OD, 498 YD and 556 RD coconut fruits were examined for the premature-nut-fall, while in Coconut Garden, Isihor, 547 GD, 498 OD, 529 OD and 494 RD coconut fruits were examined for premature-nut-fall disease (Table 2). Premature nut fall disease incidence in the 2021-2023 growing season. During the growing season (Apr 2021 - Mar 2022), in NIFOR Main Station, PDI of premature-nut-fall was 7.7%, while PDI of premature-nut-fall was 6.2% in Coconut Garden, Isihor (Table 1). During the growing season (Apr 2022 - Mar 2023), in NIFOR Main Station, PDI of premature-nut-fall was 8.8%, while PDI of premature-nut-fall was 6.2% in Coconut Garden, Isihor (Table 1).

Table 1. Coconut fruit population and premature nut fall incidence in both locations and growing seasons.

Location	Year (growing season)	Varieties	Number of coconut fruits sampled	No of premature-nut-fall	PDI
NIFOR Main Station	(2021). - 2022	GD, OD, YD, RD	1922	148	7.7
	2022 - 2023	GD, OD, YD, RD	2536	224	8.8
Coconut Garden	2021 - 2022	GD, OD, YD, RD	2238	138	6.2
	2022 - 2023	GD, OD, YD, RD	2068	129	6.2

Key: ¹GD (Green Dwarf), OD (Orange Dwarf), YD (Yellow Dwarf), RD (Red Dwarf), PDI (Percentage Disease Incidence).



Key: GD: Green Dwarf, OD: Orange Dwarf, YD: Yellow Dwarf, RD: Red Dwarf, PDI: Percentage Disease Incidence.

Figure 1. PDI of premature nut fall across coconut varieties in both locations and growing seasons.

Incidence of premature nut fall across coconut variety in 2021-2023 growing season During the growing season (Apr 2021 - Mar 2022): in NIFOR Main Station, PDI was 9.9% GD, 7.0% OD, 5.9% YD, 6.9% RD; in Coconut Garden, Isihor, PDI was 7.0% GD, 6.1% OD, 5.2% YD, 6.2% RD (table 2). During the growing season (Apr 2022 - Mar 2023): in NIFOR Main Station, PDI was 9.8% GD, 9.1% OD, 2.0% YD, 7.6% RD; in Coconut Garden, Isihor PDI was 8.4% GD, 6.8% OD, 5.1% YD, 4.7% RD (Figure 1 and Table 2).

Table 2. Premature-nut-fall incidence across coconut varieties in both locations and seasons.

Location	Growing season	Varieties	No. of coconut fruits sampled	No. of premature-nut fall	PDI
NIFOR Main Station	2021 - 2022	GD	638	63	9.9
		OD	487	34	7.0
		YD	422	25	5.9
		RD	375	26	6.9
	2022 - 2023	GD	921	91	9.8
		OD	561	51	9.1
		YD	498	40	2.0
		RD	556	42	7.6
Coconut Garden	2021 - 2022	GD	627	44	7.0
		OD	545	33	6.1
		YD	500	26	5.2
		RD	566	35	6.2
	2022 - 2023	GD	547	45	8.2
		OD	498	34	6.8
		YD	529	27	5.1
		RD	494	23	4.7

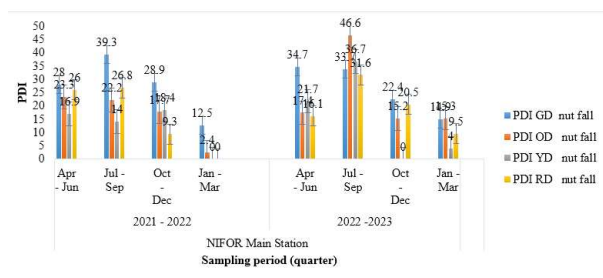
Key: ¹GD (Green Dwarf), OD (Orange Dwarf), YD (Yellow Dwarf), RD (Red Dwarf), PDI (Percentage Disease Incidence).

Incidence of premature-nut-fall across coconut varieties, sampling period (quarter) in both locations and growing seasons During the growing season (April 2021 - March 2022); in NIFOR Main Station; GD: the 3rd quarter (Jul – Sep) has a high PDI of 39.3%, while the 1st quarter (Jan – Mar) has a low PDI of 12.5%; OD: the 2nd quarter (Apr – Jun) has a high PDI of 23.3%, while the 1st quarter (Jan – Mar) has a low PDI of 2.4%; YD: the 4th quarter (Oct – Dec) has a high PDI of 18.4%, while the 1st quarter (Jan – Mar) no disease incidence was recorded for premature-nut-fall; RD: the 3rd quarter (Jul – Sep) has a high PDI of 26.8%, while the 1st quarter (Jan – Mar) no disease incidence was recorded for premature-nut-fall (Figure 2a).

In Coconut Garden, Isihor; GD: the 3rd quarter (Jul – Sep) has a high PDI of 34.6%, while the 1st quarter (Jan – Mar) has a low PDI of 4.8%; OD: the 3rd quarter (Jul – Sep) also has a high PDI of 30.3%, while the 1st quarter (Jan – Mar) has a low PDI of 4.1%; YD: the 3rd quarter (Jul – Sep) also has a high PDI of 24.6%, while the 1st quarter (Jan – Mar) no disease incidence was recorded for premature-nut-fall; RD: the 3rd quarter (Jul – Sep) also has a high PDI of 30.1%, while the 4th quarter (Oct – Dec) has a low PDI of 4.9% (figure 2b). During the growing season (April 2022 - March 2023); in NIFOR Main Station; GD: the 2nd quarter

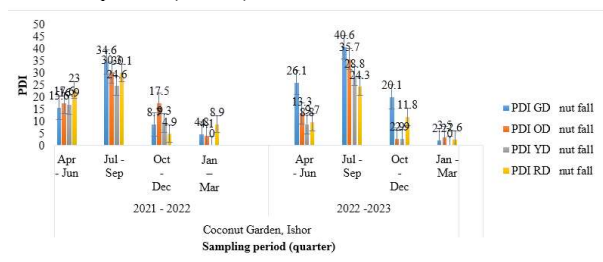
(Apr – Jun) has a high PDI of 34.7%, while the 1st quarter (Jan – Mar) has a low PDI of 14.9%; OD: the 3rd quarter (Jul – Sep) has a high PDI of 46.6%, while the 4th quarter (Oct – Dec) has a low PDI of 15.2%; YD: the 3rd quarter (Jul – Sep) also has a high PDI of 36.7%, while the 4th quarter (Oct – Dec) no disease incidence was recorded for premature-nut-fall; RD: the 3rd quarter (Jul – Sep) also has a high PDI of 31.6%, while the 1st quarter (Jan – Mar) has a low PDI of 9.5% (figure 2a).

In Coconut Garden, Isihor; GD: the 3rd quarter (Jul – Sep) has a high PDI of 40.6%, while the 1st quarter (Jan – Mar) has a low PDI of 2.2%; OD: the 3rd quarter (Jul – Sep) also has a high PDI of 35.7%, 4th quarter (Oct – Dec) has a low PDI of 2.9%; YD: the 3rd quarter (Jul – Sep) also has a high PDI of 28.8%, while 1st quarter (Jan – Mar) no disease incidence was recorded for premature-nut-fall; RD: the 3rd quarter (Jul – Sep) also has a high PDI of 24.3%, while the 1st quarter (Jan – Mar) has a low PDI of 2.6% (Figure 2b).



Key: 'GD (Green Dwarf), OD (Orange Dwarf), YD (Yellow Dwarf), RD (Red Dwarf), PDI (Percentage of Disease Incidence).

Figure 2a: Percentage of disease incidence of premature-nut-fall across varieties, growing seasons, and sampling period (month) in NIFOR Main Station.



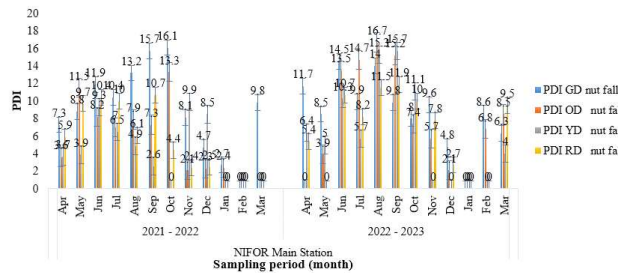
Key: 'GD (Green Dwarf), OD (Orange Dwarf), YD (Yellow Dwarf), RD (Red Dwarf), PDI (Percentage of Disease Incidence).

Figure 2b. Percentage of disease incidence of premature-nut-fall across varieties, growing seasons, and sampling period (month) in Coconut Garden, Isihor.

Incidence of premature-nut-fall across coconut varieties, sampling period (month) in both locations and growing seasons During the growing season (April 2021 – March 2022); in NIFOR Main Station; GD: Oct has a high PDI of 16.1%, while in Feb no disease incidence was recorded for premature-nut-fall; OD: Oct also has a high PDI of 13.3%, while in Feb and Mar no disease incidence was recorded for premature-nut-fall; YD: Nov has a high PDI of 9.9%, while in Oct, Jan, Feb, and Mar no disease incidence was recorded for premature-nut-fall; RD: Sep has a high PDI of 10.7%, while in Jan, Feb, and Mar no disease incidence was recorded for premature-nut-fall (Figure 3a).

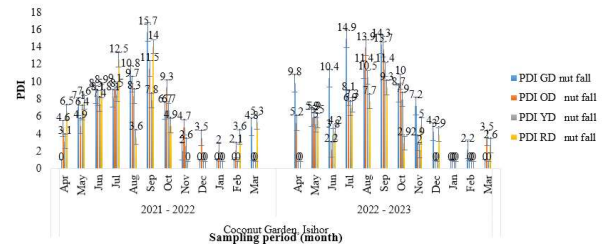
In Coconut Garden; GD: Sep has a high PDI of 15.7%, while in Apr, Jan, and Feb no disease incidence was recorded for premature-nut-fall; OD: Sep also has a high PDI of 11.5%, while in Mar no disease incidence was recorded for premature-nut-fall; YD: Jul has a high PDI of 8.5%, while in Dec, Jan, Feb, and Mar no disease incidence was recorded for premature-nut-fall; RD: Sep has a high PDI of 14.0%, while in Nov, Dec, and Jan no disease incidence was recorded for premature-nut-fall (figure 3b). While during the growing season (April 2022 – March 2023); in NIFOR Main Station; GD: Jun has a high PDI of 14.5%, while in Jan no disease incidence was recorded for premature-nut-fall; OD: Aug has a high PDI of 16.7%, while in Jan no disease incidence was recorded for premature-nut-fall; YD: Sep has a high PDI of 15.7%, while in Nov, Dec, Jan, and Feb no disease incidence was recorded for premature-nut-fall; RD: Sep also has a high PDI of 11.9%, while in May, Jan, and Feb no disease incidence was recorded for premature-nut-fall (figure 3a).

In Coconut Garden; GD: Jul has a high PDI of 14.9%, while in Jan and Mar no disease incidence was recorded for premature-nut-fall; OD: Aug has a high PDI of 13.9%, while in Dec, Jan, and Feb no disease incidence was recorded for premature-nut-fall; YD: Sep has a high PDI of 11.4%, while in Apr, Dec, Jan, and Feb no disease incidence was recorded for premature-nut-fall; RD: Sep also has a high PDI of 9.3%, while in Apr, Jan, and Feb no disease incidence was recorded for premature-nut-fall (Figure 3b).



Key: GD (green dwarf), OD (orange dwarf), YD (yellow dwarf), RD (red dwarf), PDI (percentage disease incidence).

Figure 3a. Percentage of disease incidence of premature-nut-fall across varieties, growing seasons, and sampling period (month) in NIFOR Main Station.



Key: GD (green dwarf), OD (orange dwarf), YD (yellow dwarf), RD (red dwarf), PDI (percentage disease incidence).

Figure 3b. Percentage of disease incidence of premature-nut-fall across varieties, growing seasons, and sampling period (month) in Coconut Garden, Isihor.

Table 3. Coconut premature-nut-fall of different ages (sizes) in two study locations.

Location	Fruit age (size)	No. nut fall samples	Nut fall yielding fungi		Nut fall not yielding fungi	
			No.	%	No.	%
NIFOR Main Station	1-month-old (Button nut)	113	106	93.81	7	6.19
	2-3 month-old (Fistula nut)	226	216	95.58	10	4.42
	4-7 month-old (Tender nut)	28	26	92.86	2	7.14
	8-12-month-old (Mature nut)	5	5	100.00	0	0.00
	Total	372	353	94.89	19	5.11
Coconut Garden, Isihor	1-month-old (Button nut)	72	69	95.83	3	5.63
	2-3 month-old (Fistula nut)	149	145	97.32	4	2.68
	4-7 month-old (Tender nut)	39	35	89.74	4	10.27
	8-12 month-old (Mature nut)	7	7	100.00	0	0.00
	Total	267	256	95.88	11	4.12
Grand total		639	609	95.31	30	4.69

Fungal isolates from the fruit and floral parts of the coconut in both locations. The tables below provide the results of fungi isolated from fruits and floral parts of the coconut in both locations.

Table 4a. Fungal isolates from fruit and floral parts of coconut in NIFOR Main Station.

Location	Parts of a coconut	Fungal isolates	Mycelium colour after 5 days on PDA
NIFOR Main Station	Fruit: Tip (Point of attachment of fruit to spikelet (sample taken from fruit))	<i>Botryodiplodia theobromae</i> <i>Fusarium sp.</i> <i>Rhizopus stolonifer</i> <i>Pestalotiopsis palmarum</i>	Dark grey Pinkish Greyish White velvety
	Mesocarp	<i>Botryodiplodia theobromae</i>	Dark grey
	Endocarp	<i>Botryodiplodia theobromae</i>	Dark grey
	Endosperm (coconut meat/water)	<i>Botryodiplodia theobromae</i>	Dark grey
	Spikelet: Point of attachment of fruit to spikelet (sample taken from spikelet)	<i>Botryodiplodia theobromae</i> <i>Fusarium sp.</i> <i>Rhizopus stolonifer</i> <i>Pestalotiopsis palmarum</i>	Dark grey Pinkish Greyish White velvety
	Point of attachment of spikelet to floret (sample taken from spikelet)	<i>Botryodiplodia theobromae</i> <i>Fusarium sp.</i> <i>Rhizopus stolonifer</i>	Dark grey Pinkish Greyish
	Inflorescence (Floret): (Point attachment of spikelet to floret (sample taken from floret))	<i>Botryodiplodia theobromae</i> <i>Fusarium sp.</i> <i>Rhizopus stolonifer</i>	Dark grey Pinkish Greyish
	Calyx:	<i>Botryodiplodia theobromae</i> <i>Fusarium sp.</i> <i>Verticillium sp.</i>	Dark grey Pinkish Light yellow

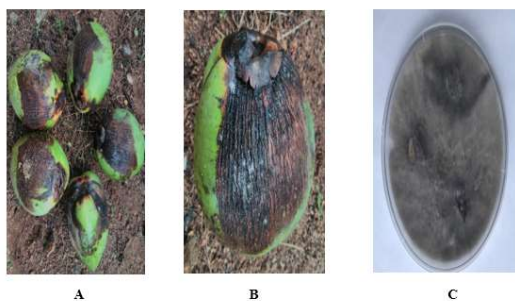


Figure 4. (A) Cluster of fallen nuts of mature GDC with extensive rot; (B) Fallen nut (premature-nut-fall) of mature GDC with extensive rot; (C) 7-day-old *B. theobromae* isolated from GDC after direct plating with grey color.

Table 5. Fungal isolates from fruit and floral parts of coconut in Coconut Garden, Isihor.

Location	Parts of a coconut	Fungal isolates	Mycelia colour after 5 days on PDA
Coconut Garden, Isihor	Fruit: Tip (Point of attachment of fruit to spikelet (sample taken from nut))	<i>Botryodiplodia theobromae</i> <i>Verticillium sp.</i> <i>Aspergillus sp.</i> <i>Pestalotiopsis palmarum</i>	Dark grey Light yellow Greenish White velvety
	Mesocarp	<i>Botryodiplodia theobromae</i>	Dark grey
	Endocarp	<i>Botryodiplodia theobromae</i>	Dark grey
	Endosperm (coconut meat/water)	<i>Botryodiplodia theobromae</i>	Dark grey
	Spikelet: Point of attachment of nuts to spikelet (sample taken from spikelet)	<i>Botryodiplodia theobromae</i> <i>Verticillium sp.</i> <i>Pestalotiopsis palmarum</i>	Dark grey Light yellow White velvety
	Point of attachment of spikelet to floret (sample taken from floret)	<i>Botryodiplodia theobromae</i> <i>Verticillium sp.</i> <i>Pestalotiopsis palmarum</i>	Dark grey Light yellow White velvety
	Inflorescence (Floret): (Point attachment of spikelet to floret (sample taken from floret))	<i>Botryodiplodia theobromae</i> <i>Verticillium sp.</i> <i>Pestalotiopsis palmarum</i>	Dark grey Light yellow White velvety
	Calyx:	<i>Botryodiplodia theobromae</i> <i>Aspergillus sp.</i> <i>Pestalotiopsis palmarum</i>	Dark grey Greenish White velvety

3.2. Discussions

Thus far, the incidence of premature-nut-fall disease in coconuts has been sparsely studied in coconut plantations. In Nigeria, the study provides a first report on the intensive field data of fungi associated with the premature-nut-fall disease of coconut, and the variability of the disease over time in two major coconut-growing areas in Ovia-North-East Local Government Area, Edo State, Nigeria. According to Rajapakse *et al.* (1995), three significant factors, namely microbial infections, insect infestation, and physiological reasons, were identified as predominant causes for premature-nut-fall disease of coconut. Light or dark brown patches identified microbes (especially fungi infections on coconut fruits) with or without a water-soaked appearance and the presence of mycelium in the

perianth region and inner tissues, and isolations were performed on discolored infected tissues. For insects: attacks were identified by their presence, feeding signs, larval tunnels, webbing, and scraping marks on the mature and immature nuts. Insect infestation of the coconut fruits was rare in the study.

For physiological reasons, falls of mature and immature nuts due to physiological disorders were identified by their healthy appearance with or without characteristic uniform browning of the nuts. Based on these facts, the study categorized premature-nut-fall into "nut-yielding fungi, and " nut-not-yielding fungi". The percentage incidence of nut-yielding fungi (compared to nut-not-yielding fungi) was over 94%, revealing that the premature nut fall is primarily a result of fungi infection. Before now, information on the incidence of premature-nut-fall disease caused by *B. theobromae* was very scarce (Dheepa *et al.*, 2018; Gunasekaran & Srinivasan, 2000; Rajapakse *et al.*, 1995; Sulladmath & Shanthappa, 1979). It was not considered a serious problem until recently, when fruit-rot and premature-nut-fall diseases due to the pathogen increased considerably in coconut-producing areas (Venugopal & Chandra Mohanan, 2006). The study provides information on the incidence of the premature-nut-fall disease of coconut concerning *Botryodiplodia theobromae* and other fungi associated with the disease over two overlapping growing seasons (2021 - 2022 and 2022 - 2023).

The percentage of disease incidence (PDI) of coconut in both growing seasons varies from 6.2 to 8.8% in both locations (Tables 1 and 2). During the growing season (Apr 2021 - Mar 2022), in NIFOR Main Station, PDI of premature nut fall was 7.7%, while during the growing season (Apr 2022 - Mar 2023), in NIFOR Main Station, PDI of premature nut fall was 8.8%. However, the PDI of premature nut fall in Coconut Garden, Isihor was 6.2% in both growing seasons (Table 1). The PDI in both growing seasons was higher in NIFOR Main Station than in Coconut Garden, Isihor. In NIFOR Main Station, the PDI was higher in the 2022 – 2023 growing season than in the 2021 - 2022 growing season (Table 1). In the study, the PDI of coconut's premature nut fall disease reveals high variability within and between both locations across the growing seasons. Cumulatively, the Green Dwarf (GD) coconut has a higher PDI of premature nut fall across the growing season in both locations (Table 2).

The incidence of premature nut fall across coconut varieties varies from 2.0 to 9.9% in both locations and growing seasons (Table 2), which reveals a high variability of premature nut fall disease across coconut varieties in both locations and growing seasons. Coconut varietal premature-nut-fall incidence across the sampling period

(quarter) in both growing seasons and locations varies from 2.2 to 46.6%. In both locations, PDI of premature-nut-fall was higher in the 3rd quarter (Jul - Sep) across all varieties in both growing seasons except for YD and GD coconut that the PDI of premature-nut-fall was higher in the 4th quarter (Oct - Dec) of the 2021 – 2022 growing season and the 2nd quarter (Apr – Jun) of 2022 – 2023 growing season respectively (figure 2a and b). The study reveals a high level of variability in the PDI of premature-nut-fall across the sampling period (quarter) in both growing seasons and locations. Coconut varietal premature-nut-fall incidence across the sampling period (month) in both growing seasons and locations varies from 2.0 to 16.7%. During the growing season 2021 – 2022, in NIFOR Main Station, the Green Dwarf (GD) coconut has a higher PDI (16.1%) of premature-nut-fall in Oct, while in Coconut Garden, Isihor, the green dwarf (GD) coconut also has a higher PDI (15.7%) of premature-nut-fall in Sep. During the growing season 2022 – 2023; in NIFOR Main Station, the Orange Dwarf (OD) coconut has a higher PDI (16.7%) of premature-nut-fall in Aug, while in Coconut Garden, Isihor, the green dwarf (GD) coconut has a higher PDI (14.9%) of premature-nut-fall in Jul (figure 3a and b). The study also reveals a high variability in the PDI of premature-nut-fall across the sampling period (month) in both growing seasons and locations.

Premature nut fall incidence among different fruit age (size) collected from the two locations; In NIFOR Main Station, out of the 372 coconut samples that experienced premature-nut-fall, fungi were isolated from 353 (94.89%) coconut samples, while no fungi were isolated from 19 (5.11%) coconut samples. In Coconut Garden, Isihor, out of the 245 coconuts that experienced premature-nut-fall, fungi were isolated from 235 (95.92%) coconut samples, while no fungi were isolated from 10 (4.08%) coconut samples (Table 3). Thus, in both locations, the high percentage of fungi isolated from fallen nuts indicates the role of fungal pathogens in premature-nut-fall disease of coconut. The study agrees with Venugopal and Chandra Mohanan (2006), who reaffirm the role of fungi in premature nut fall disease of coconut. Furthermore, in both locations, among the fruit age (size), the fistula size (2-3 month-old nuts) is most affected by premature-nut-fall diseases, while the mature size (10-12 month-old nuts) is less affected by the diseases (Table 3). In both locations, over 94% of fungi were isolated from the internal tissues of diseased fruits with signs and symptoms of premature-nut-fall (fallen nuts) (Table 3). In both locations, fungi were not isolated from some of the fallen nut samples collected. This may be attributed to other factors such as insect pest infestation or physiological or environmental factors.

The fungi isolated from the internal tissues of fallen nuts were mainly *B. theobromae*. In contrast, fungi isolated from the floral parts of coconut include: *Aspergillus sp.*, *B. theobromae*, *Fusarium sp.*, *Neodeighonia licuriensis*, *Pestalotiopsis palmarum*, *Rhizopus stolonifera*, and *Verticillium sp.* In both locations, isolation of *B. theobromae* from diseased coconut fruits indicates that the fungus is the pathogen responsible for the premature-nut-fall disease of coconut. This study agrees with Venugopal and Chandra Mohanan's (2006) work. The isolation of other fungal flora parts of the coconut was minimal compared to *B. theobromae*. In addition, *B. theobromae* was solely isolated from the mesocarp, endocarp, and endosperm (coconut meat and water) of fallen nuts with signs of rot.

4. CONCLUSIONS

This intensive study on the premature-nut-fall disease of coconut has revealed the prevalence of *B. theobromae* responsible for the disease. Previously, this pathogen was considered a weak pathogen, but over time, research has shown that the pathogen is a serious pathogen causing considerable loss to coconut breeders and farmers. Hence, there is a need to develop appropriate disease-control interventions towards this pathogen and other fungi isolated from the floral parts of the coconut. In the long run, controlling the premature-nut-fall disease will help improve coconut yield.

REFERENCES

- Adkins, S., M. Foale & Samosir, Y. (eds.). (2006). Coconut revival: new possibilities for the 'tree of life'. In *Proceedings of the International Coconut Forum*. Australian Centre for International Agricultural Research, Canberra, ACT, pp. 1–103.
- Bourke, R.M. & Harwood, T. (Eds.). (2009). Food and Agriculture in Papua New Guinea. Australian National University. Australia, pp. 327.
- Dheepa, R., C. Goplakrishnan, A. Kamalakannan, S. Nakkeeran, C.A. Mahalingam, & J. Suresh. (2018). Coconut nut rot disease in India: Prevalence, characterization of pathogen and standardization of inoculation techniques. *Int.J.Curr.Microbiol.App.Sci.* 7(2): 2046–2057.
- Ekhurutomwen, E.O., M. E. Udoh, & D.O. Esiegbuya. (2017). Preliminary survey study on coconut fruit rot disease incidence in Badagry, Lagos State, Nigeria. NIFOR Annual Report, 56: 157–158.
- FAO. (2014). *FAO Stat. Vol. 2014*. Rome: Food and Agriculture Organisation of the United Nations.
- Gurr, G.M., A.C. Johnson, G.J. Ash, B.A.L. Wilson, M.M. Ero, C.A. Pilotti, C.F. Dewhurst, & M.S. You. (2016). Coconut lethal yellowing diseases: A phytoplasma threat to palms of global economic and social significance. *Front. Plant Sci.* 7: 1521.
- Gunasekaran, M. and N. Srinivasan. (2000). Involvement of *Lasiodiplodia theobromae* (Pat) Griffon and Maublin fruit rot of coconut in India. *Trop. Agric.* 77: 123 – 125.
- Ojomo, E.E., I.J. Osaigie, and M.E. Udoh. (2010). Fungal diseases of coconut palm in Nigeria. *Nig. J. of Palms and Oil Seeds.* 17: 52–61.
- Phipps, P.M. & D.M. Porter. (1998). Collar rot of peanut caused by *Botryodiplodia theobromae*. *Plant Dis.* 82: 1205–1209.
- Quillec, G., J.L. Renard and H. Chesquiere. (1984). *Phytophthora heveae* of coconut: Role in bud rot and nut fall. *Oleagineux*, 39 (10); 483–485.
- Rajapakse, C.N.K., H.T.R. Wijesekara & A. H. Norman. (1995). A preliminary study on immature nut fall of coconut with reference to pest damage. *Cocos*, 10: 53–54.
- Rangaswami, G., T.K. Kandasamy, & K. Ramasam. (1975). *Pleurotus sajor caju* (Fr.). Singer-protein rich nitrogen fixing mushroom fungi. *Current sci.*, 44: 403–444.
- Sudhakara, K. (1991). Button shedding and premature nut fall in the coconut. *Indian Coco. J.*, 22(5): 13–20.
- Sulladmath, V.V. & P.B. Shantappa. (1979). Nut fall in coconut associated with *Botryosphaeria rhodina* (Berks and Curt pv. Arx). *Curr. Res.*, 8:13.
- Venugopal, S. & ChandraMohanan, R. (2006). Role of fungi in fruit rot and immature nut fall of coconut. *Cord*, 22 (2): 1 – 8.