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First Report of *Curvularia pseudobrachyspora* Causing Leaf Spots Disease on Coconut (*Cocos nucifera* L.) Seedlings in Ghana

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Abstract

Coconut (*Cocos nucifera* L.) is an important oilseed and a multipurpose perennial plantation crop. It is mostly grown in humid and coastal areas of Ghana. In June 2019, leaf spot disease was observed on coconut seedlings in 10 coconut growing Districts in Ghana. The Initial symptoms appeared as elliptical, brown-dark-red lesions, 4 - 6 mm on affected leaves. Lesions reached 2.0 cm and gradually developed into spindly, dark brown spots with a light tan centre. A study including survey and laboratory work was carried out to assess disease prevalence and to identify the causal agent of the disease on coconut seedlings, in order to formulate effective management strategies against it. A total of 250 symptomatic leaves were picked from ten selected Districts for laboratory analysis. Additionally, the ribosomal internal transcribed spacer (ITS) region and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) of the isolated pathogen were amplified using ITS1 and ITS4, gpd1 and gpd2 primers respectively. The disease incidence peaked at 95%. Fungal colonies on PDA grew to 50 - 70 mm in diameter in one week at a temperature of 28˚C ± 1˚C with an even to undulating, immersed striated, mycelium; aerial mycelium being dark velvety green and sometime woolly-cottony. Acervuli formed on the aerial mycelium and contained black powderish conidial masses. Hyphae characteristics were similar to that of *Curvularia pseudobrachyspora*. Pathogenicity tests were done following Koch's postulate. For molecular confirmation, the combined ITS (MT075719) and GAPDH (MT075720) sequences were compared with published sequences of 52 *Curvularia* isolates and eight *Bipolaris* isolates using phylogenetic analysis. This is the first report of *C. pseudobrachyspora* as a causal agent of leaf spot on coconut seedlings in Ghana and possibly Africa but the pathogen has been reported on other crops in China, Florida and India.

Keywords

Leafspots, *Curvularia* sp., Pathogen, Phylogenetic Analysis

1. Introduction

The coconut palm (*Cocos nucifera* L.) is one of the world's most important oil-producing plants second to oil palm and provides a substantial export income for many tropical countries, as well as food, drink, fuel and shelter [1]. It is reported that the coconut trade is a major source of export revenue thus, contributing more than 50% of export income as well as accounting for about 1.5% of Gross National Product (GNP). Generally, global coconut production continues to indicate a relatively flat rate of growth after attaining approximately 62 million metric tonnes in 2007 [1] [2].

In Ghana, the coconut subsector used to be limited to the coastal areas where about 80% of the total land area under coconut cultivation, estimated at 44,000 ha, is found [3]. Currently, due to increased demand for coconut products globally and the favourable political climate created for the cultivation of coconut through the government flagship programme of "Planting for Export and Rural Development" the subsector is rapidly expanding into the hinterland across all the regions of Ghana which have suitable climatic conditions for coconut cultivation. This rapid expansion has led to high demand for coconut planting materials. Quality planting material free of diseases is critical for this expansion.

According to Rawal [4], coconut production is estimated to increase by at least 28% globally if the crop is protected against various diseases at the nursery. In Ghana, the main nursery disease currently of concern to coconut growers is leaf spot disease.

The disease is characterised by small, circular brown to black spots with tan/necrotic centre on leaves in the nursery and very young plants. Spots gradually turn brown, with the centre of the spot drying up with a sunken appearance. The disease turns serious in neglected ill-drained gardens and in nurseries raised under heavy shade [5].

Aside from reports and control measures to combat some diseases on coconut, very little is known about the cause of leaf spots on coconut seedlings in Ghana. Appropriate disease diagnosis and management strategies are yet to be identified. This work, therefore, sought to identify effective management strategies against the disease through assessing disease prevalence, identification and confirmation of causal agent of the disease on coconut seedlings in Ghana.

2. Materials and Methods

2.1. Study Site

The study was carried out in ten selected coconut growing Districts in Ghana with farmers' reports on leaf spot disease occurrences as the criteria for selection

of the Districts. The areas surveyed include Kwaebibirim, Denkyembour, West Akim, East Akim, Bonsu, New Akyem Tafo, Keta, Aseibu, Axim and Eji-su-Besease. These areas lie on latitude 001˚45West and longitude 0.6˚00 North and are located within the coastal and semi-deciduous forest zone of Ghana. The areas are characterized by double maxima rainfall pattern followed by a prolonged dry season. The minimum temperature during the first three months of the study period (April to June 2019) ranged between 21.6˚C and 24˚C and maximum varied between 32.3˚C and 34.1˚C. The relative humidity varied from 50% to 70%.

2.2. Disease Assessment

Disease incidence was assessed as percentage of plants infected with at least one leaf spot or visible symptomatic number of coconut seedlings found in the nurseries per plot using disease index/sheet. A total of 5000 coconut seedlings were assessed using 500 seedlings per area/District.

2.3. Percentage Disease Intensity and Severity

Disease severity was evaluated using the method described by Lekete *et al.* [6] and Khan and Hosain [7]. The scale used is detailed in **Table 1**.

Percentage disease intensity (PDI) was calculated using the formula given below.

$$\%\ \text{Percentage disease intensity}\left(\text{PDI}\right)=\frac{\text{Sum of the score}}{\text{Number of Rating}\times\text{maximum score per plot}}\times 100$$

Twenty-five leaflets of disease symptoms from each location were collected into 229 mm × 324 mm sterilized Ziploc bags, labelled and brought to the Plant Pathology laboratory of CSIR-Oil Palm Research Institute (OPRI), Kusi for further clinical analysis and examination. Possible insect vector involvement was also checked.

2.4. Disease Isolation

Disease isolation was done following a method described by Lekete *et al.* [6]. Symptomatic leaf samples were surface washed thoroughly under running tape water and later cut into 1 cm pieces, surface sterilized with 10% of Sodium-Hypo-Chloride

Table 1. Scale used for scoring coconut leaf spot disease severity.

% leaflet area covered	Severity Score
0	0
1 - 5	1
5.1 - 12	2
12 - 25	3
25.1 - 50	4
50.1 and above (>50)	5

(NaOCl) for 30 sec and transferred into 15% Hydrogen Peroxide solution (H_2O_2) and serially washed in three changes of sterile distilled water, blotted dry, and then placed excised pieces of leaves from the lesion margin onto Water Agar and then potato dextrose agar (PDA). Plates were incubated at 28˚C ± 1˚C for 4 days.

2.5. Morphological Identification

Isolates from various damaged symptoms found in coconut leaflets were examined under microscope with 40× magnification after staining. Fungi were identified by conventional method based on the colony characters, nature of growth, spores shape, size, colour and septation of different fungal parts.

2.6. Molecular Identification and Confirmation

Fresh mycelium (0.6 g) was scraped from the surface of a colonised Potato Dextrose Agar plate and transferred to 1.5 ml micro centrifuge tubes. The mycelium was ground for 3 - 5 min with a sterilised glass pestle after adding 600 µl of pre-heated (60˚C) 2 × CTAB extraction buffer (2% (w/v) CTAB, 100 mM Tris–HCl, 1.4 M NaCl, 20 mM EDTA, pH8.0) and 0.2 g sterilised quartz sand. The solution was incubated at 60˚C with a gentle swirling. The mixture was subsequently centrifuged at 12,000 rpm for 15 mins at 25˚C followed by chloroform iso-amyl extraction repeatedly. DNA was precipitated with isopropanol and centrifuged at 12,000 rpm for 15 mins at 25˚C. The precipitate was treated with 70% ethanol centrifuged at 12,000 rpm for 3 - 5 min at 25˚C. DNA was dried under a regular air flow for 15 mins, re-suspended in 70 µl TE buffer and stored in −20˚C.

The identity of the isolates was confirmed via sequence analysis of the internal transcribed spacer (ITS) gene amplified using primers ITS1/ITS4 [8], glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene amplified using gpd1/gpd2 [9], and translation elongation factor 1-alpha (EF1a) gene amplified using 983F/2218R [10]; sequence data were deposited in GenBank as (MT075719 and MT075720).

2.7. Artificial Inoculation

It is believed that foliar diseases including coconut leaf spots are mostly reported from neglected nurseries raised under shed. Therefore, to confirm pathogenicity test, 24 bags of two-year old apparently healthy coconut seedlings raised at CSIR-OPRI nursery were selected for pathogenicity test. Four seedlings were infected with each of five fungal isolates while leaves/seedlings sprayed with sterile distil water were set aside as control. The exercise took place behind CSIR-OPRI plant pathology laboratory. Surfaces of three leaflets of each selected seedlings were disinfected with 70% ethanol [11]. Then 10-µl conidia suspension (1×10^5 spores/mL) of 7-day old culture of the isolated fungus was sprayed on the cleaned leaves. The entire setups were covered with transparent polythene bags to artificially induce symptoms. The seedlings were kept in an improvised green house at temperature of 29˚C - 32˚C for 14 days. The pathogen was re-isolated

from inoculated leaves. To validate the results, pathogenicity test was repeated three times but the same results were obtained in each case.

2.8. Experimental Data Analysis

The data on different parameters were compiled and analyzed using R-Stat version 2018.

3. Results

3.1. Field Observation and Examination

Preliminary investigation on seedling diseases of coconut in different localities including the five selected coconut nursery communities visited revealed that leaf spot was the most common disease. Detailed examination and disease assessment performed on infected coconut seedlings showed high incidence of leaf spot disease. A total of 4755 (95.1%) out of the 5000 infected seedlings examined showed leaf spot with disease incidence significantly ($P < 0.005$) higher during the wet season. Disease severity score ranged between 3 and 5 in the selected study plots (an epidemic situation) (**Figure 1**). Incidence of leaf spot disease in coconut seedlings varied significantly depending on season and location.

The highest incidence (95.1%) of leaf spot was observed in May, 2019 at Kwaebibirim, followed by Denkyembour (80.3%); the lowest (28.0%) was observed in June, 2019 at Ejisu Juaben District.

3.2. Material Examined

Leaf spot disease symptom on coconut first appeared as fusiform or elliptical, brown-red, with light tan center and then dark brown (**Figure 1**). The lesions gradually expand across the leaf vein to the leaf apex (**Figure 2**) at the later stage of the infection.

3.3. Diagnostic Results

Portion of symptomatic leaf samples were collected and cultured on PDA medium. Mycelial growth started emerging from the infected leaf portion from the third day (**Figure 3(A)**). For the purpose of identification, a portion of the emerging mycelial growth was sub-cultured. Pure culture of fungi thus obtained was used to identify the respective fungus using standard taxonomic references.

The percentage occurrences of most common fungi isolated in this study were *Curvularia* sp. (78.0%), *Pestaloptiopsis* sp. (8.4%), *Bipolaris* sp. (6.8%) and *Fusarium* sp. (4%) with the remaining 2.8% being saprophytes. The suspected fungi identified to cause coconut leaf spot disease are listed **Table 2**.

3.4. Morphology and Molecular Identification

Asexual morphology on PDA: Fungal colonies on PDA grew to 50 - 70 mm diameter in one week at a temperature of 28˚C ± 1˚C with an even to undulating,

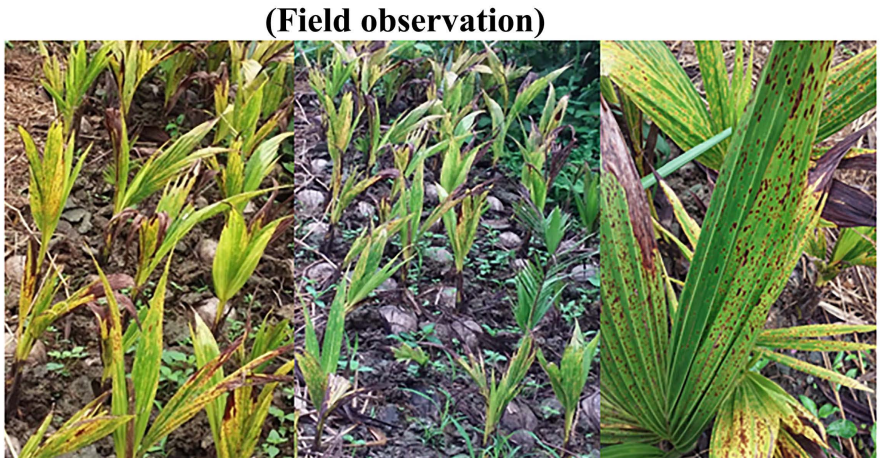

Figure 1. An Epidemic situation of leaf spots disease on coconut seedlings.

Figure 2. Symptomatic leaf sample examined.

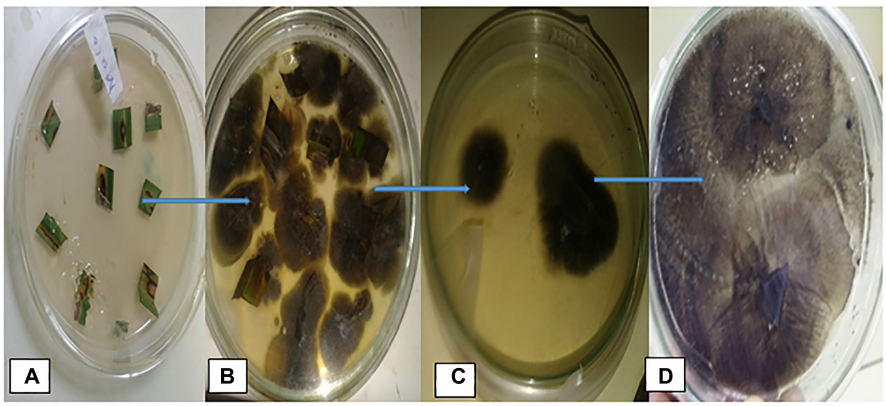

Figure 3. (A & B) Initial isolation, (C) Sub-culture in growth medium, (D) Final pure culture obtained.

striated, glabrous, villous, greenish margin; immersed stranded mycelium was dark green to black; aerial mycelium appeared dark velvety green and sometime woolly-cottony (**Figures 3(B)-(D)**). Acervuli formed on the aerial mycelium and contained black, powderish conidial masses (**Figure 3(D)**).

Table 2. Fungal isolates identified.

Leaf spot Disease Incidence		
Fungal Isolates identified based on morphology in decreasing order		
Fungal sp.	No. of occurrence	Percentage incidence (%)
Curvularia sp. (Leaf spot)	195	78.0
Pestalotiopsis sp.	21	8.4
Bipolaris sp.	17	6.8
Fusarium sp.	10	4
Others (saprophyte)	7	2.8
Total	**250**	**100**

Asexual morphology on PDA

Morphology characteristics (Micrograph): Hyphae hyaline to pale brown, branched, septate, 2 - 4 (-5) µm diam (Figure 4). Conidiophores arising singly or in groups, septate, straight or flexuous, sometimes geniculate at upper part, size of cells rarely decreasing towards apex, sometimes branched, cell walls thicker than those of vegetative hyphae, mononematous, semi- to macronematous, pale brown to brown, paler towards apex, sometimes swollen at the base, 110 - 420 × (2.5-) 3.5 - 6 (-7) µm (Figure 4 & Figure 5).

Conidiogenous cells smooth-walled, terminal or intercalary, proliferating sympodially, pale brown to brown, subcylindrical to slightly swollen, 7 - 24 (-26) × 4 - 6.5 µm. *Conidia* verruculose, mostly curved, ellipsoidal to obovoid, pale brown to brown, apical and basal cells paler than the middle cells, (3-)4-distoseptate, (16-) 21.5 - 27 (-28.5) × 8 - 14 µm; *hila* protruding, darkened, thickened, 2 - 3 (-4) µm. *Microconidiation*, *chlamydospores* and *sexual morph* not observed. Based on the symptoms and morphological (mycelial and conidial) characters above, the fungus was identified as *Curvularia pseudobrachyspora*, and confirmed through molecular amplification of internal transcribed spacer regions generated using ITS1 and ITS4. The sequencing results (Figure 6) were submitted to Genbank via National Center for Biotechnology Information (NCBI) and accession numbers are assigned as (MT075719; MT075720) respectively.

BLAST analysis demonstrated that the above sequences were 99% to 100% similar to the ITS (MH819562) and (NR164423.1) sequences of *C. pseudobrachyspora*.

3.5. Proof of Pathogenicity Test (Artificial Inoculation)

In all, a total of 20 seedlings were inoculated with four used as control treatment. After 14 days, seedlings inoculated with *Curvularia* sp. showed typical symptoms similar to those observed on the field (Figure 7(A) and Figure 7(B)). The same fungus was re-isolated from the lesions with morphological characteristics identical to the original isolated fungus (Figure 7(C)), confirming Koch's postulates. No symptoms appeared on the control leaves sprayed with sterile water (Figure 7(D)).

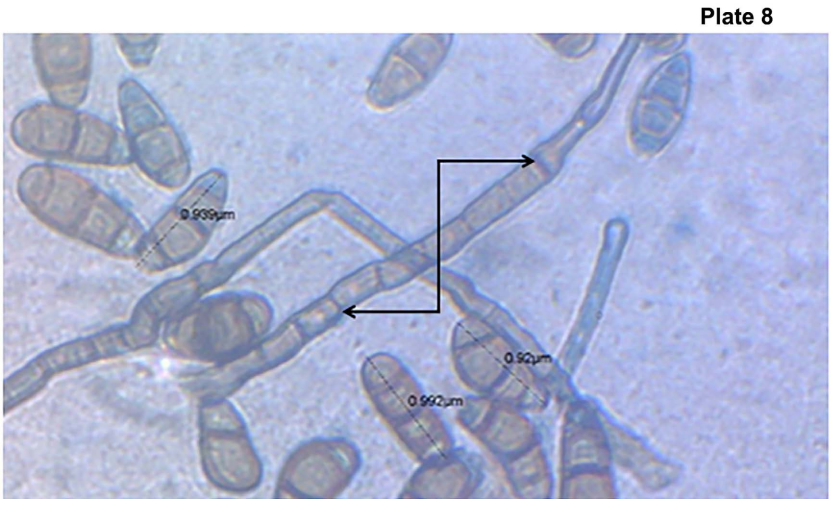

Figure 4. Septate, straight/flexuous conidiophores and conidia of *Curvularia* sp.

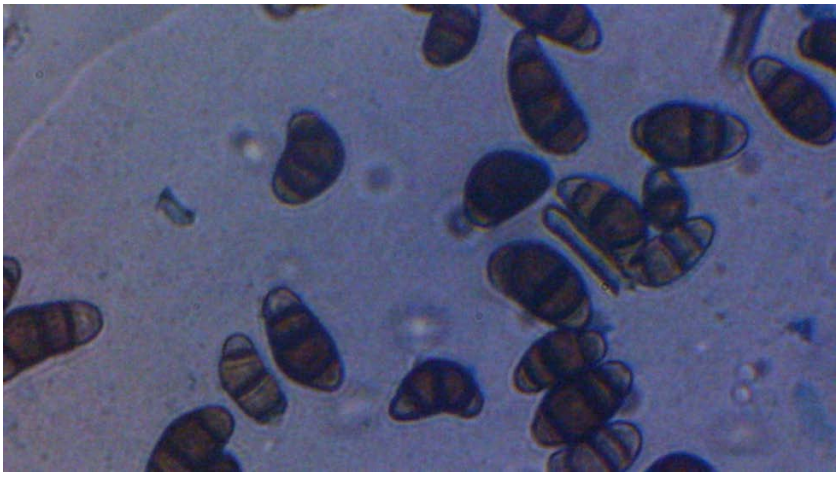

Figure 5. Curved, ellipsoidal to obovoid conidia of *Curvularia* sp.

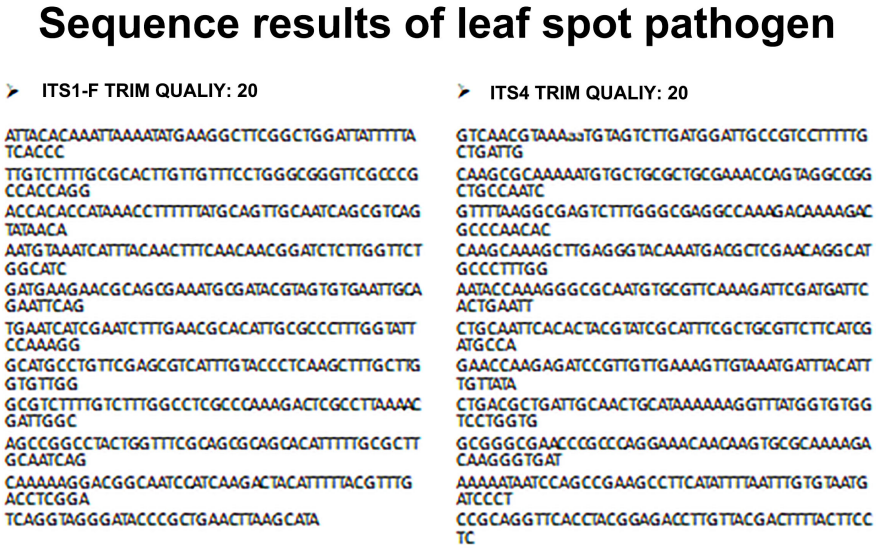

Figure 6. Sequence results of leaf spot pathogen on coconut seedlings.

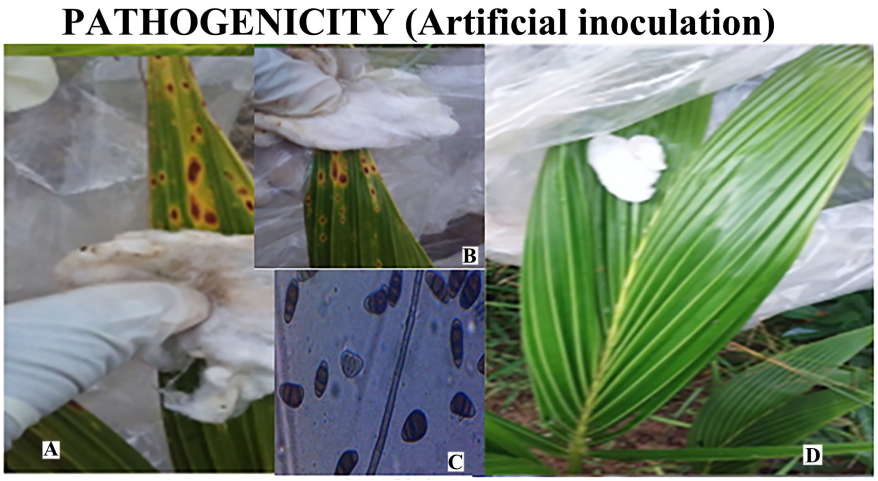

Figure 7. (A and B) Pathogenicity on coconut seedlings; (C) Conidia from re-isolated colony; (D) Control for pathogenicity test.

Pathogenicity test confirmed that *Curvularia pseudobrachyspora* could cause leaf spot disease on coconut seedlings.

4. Discussion

4.1. Field Observation and Examination

Although high rainfall and humidity are noted to favour the development and spread of most fungal diseases, in this study, high humidity and low rainfall were found to favour the disease, thus disease severity was recorded in early April to May when these conditions existed, whilst low disease severity was observed in June and July, 2019. Similar observation was made by Islam [12] on disease prevalence of leaf spot disease caused by *Pestalotia palmarum*. He also observed that coconut mainly suffered from leaf spot and bud rot disease in the nurseries.

4.2. Diagnostic Results

Fungal isolates of *Pestalotiopsis* and *Curvularia* are important phytopathogens of members of Poaceae. The diseases symptoms that these taxa cause include leaf spots, blight, melting out, root rot and foot root, among others. Several isolates belonging to both genera were isolated from these disease symptoms of different members of *Poaceae* in Ghana. Based on morphological characterization and analysis of individual micrographs of these isolates, one new fungus (*Bipolaris*) was also identified. *Bipolaris* was also a known member of *Poaceae* believed to be a causal agent of leaf blight on coconut [13]. Thus, result in mixed infections as observed on some of the infected seedlings.

4.3. Morphology and Molecular Identification

The conidia verruculose, mostly curved, ellipsoidal to obovoid, pale brown to brown, apical and basal cells paler than the middle cells, (2-)3-distoseptate, protruding, darkened, thickened, showed exact characteristics and very similar to

Curvularia pseudobrachyspora and what was identified by Manamgoda *et al.* [13], Marin-Felix *et al.* [14] and Wang *et al.* [15].

Curvularia spp. have been reported causing diseases on both annual and perennial crops including coconut, but *Curvularia pseudobrachyspora* was not previously reported on coconut. So far, this is the first report of *Curvularia pseudobrachyspora* causing leaf spot disease on coconut seedlings in Ghana. Thus, three months of investigation in 10 coconut nursery plots enabled further understanding of infection, expression of a range of symptoms, and epidemiology of the disease.

4.4. Molecular

The ribosomal internal transcribed spacer (ITS) region and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were amplified using the primers ITS1 and ITS4 [8]. The sequence results of ITS1 (MT075719) and ITS4 (MT075720) were compared with published sequence of 52 *Curvularia* isolates by phylogenetic analysis. The ceiba_1_/ceiba_2_ isolates from *Cocos nucifera* from Ghana showed 100% similarity with isolate (MH819562.1) of *Curvularia pseudobrachyspora* from Bulb Rot of Lily from China and 99.81% similarity with isolate (NR164423.1) of *C. pseudobrachyspora* from Areca palm leaf spots from Hainan Province, China [15] respectively. Surprisingly, ceiba 1 and ceiba 2 which were coconut isolates from Ghana, showed distinct characteristics from *Curvularia oryzae* strain (CBS169.53) which was isolated and identified causing leaf spot disease on coconut seedlings in China [16].

4.5. Pathogenicity

Considering the difficulties usually faced in testing pathogenicity of leaf diseases in coconut, two-year old apparently healthy coconut seedlings were used. None of the five fungi isolated apart from *Curvularia* sp. showed any infection of leaf spot. However, only *Curvularia* isolate, developed necrotic spot on the test seedlings. Out of five fungi isolated and identified only one produced the exact leaf spot symptom which was initially observed on the field. Report from early researchers [17] showed that *Pestalotiopsis* sp. could not establish pathogenicity on coconut leaves in inoculation experiments conducted in Sri Lanka.

Although Ram [18] found *Pestalotiopsis* sp. as the most prevalent coconut leaf pathogen in a survey of four sites at Sergipe province of Brazil, he could not confirm pathogenicity under the conditions tested. However, Sugata Ghose [19] who worked on coconut leaf diseases in Orissa, confirmed pathogenicity of *Curvularia* sp. to cause leaf spot in coconut after conducting three series of pathogenicity in India, thus supporting the result obtained from the present study. It was reported that most of the fungi causing leaf diseases on coconut are weak parasites, *Pestalotiopsis* and *Bipolaris* spp. which were known to cause leaf spot/grey blight symptoms on coconut in Brazil, as observed by Cardoso *et al.* [20], could not produce similar symptom on coconut leaves in this study.

5. Conclusions

Based on morphological characteristics, pathogenicity confirmation and phylogenetic sequence analysis, the causal agent of leaf spots on coconut seedlings (*Cocos nucifera*) in Ghana is identified as *Curvularia pseudobrachyspora*. This is the first report of *Curvularia pseudobrachyspora* causing leaf spots disease on coconut seedlings in Ghana and West Africa.

Pathogenicity test results showed that *Curvularia pseudobrachyspora* could infect coconut seedlings, which developed the same symptoms observed naturally in the field after artificial inoculation.

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Conflicts of Interest

With the submission of this manuscript, authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Global Trade (2019, September 18) Global Coconut Market 2019—Thailand's Imports Continue to Grow Robustly, While Domestic Production Declines. https://www.globaltrademag.com/global-coconut-market-2019-thailands-imports

[2] Shahbandeh, M. (2022) Global Coconut Production 2000-2020. https://www.statista.com/statistics/577497/word-coconut-production

[3] MoFA (Ministry of Food and Agriculture) (2018) Crop Sector Studies, Draft Report on Coconut. Ministry of Food and Agriculture, Crops Services Directorate, Accra.

[4] Rawal, R.D. (1990) Fungal and Bacterial Diseases of Fruit Crops. A Decade of Research on Diseases of Horticultural Crops under AICRIP (1980-1989). *Presented at a Group Discussion of Plant Pathologists Working in Coordinated Projects of Horticulture*, IIHR (Indian Institute of Horticultural Research), Bengaluru, 14-15.

[5] Mariau, D. (2001) Diseases of Tropical Tree Crops. IBM Publishing Co., Oxford.

[6] Lekete, E., Osekre, E.A. and Ando-Mensah, E. (2019) Report on Outbreak of Leaf Spots Disease Caused by *Pestalotiopsis* sp on Oil Palm Seedlings in Nurseries in Ghana. Short Communication. *Malaysia Journal of Microbiology*, **15**, 419-424. https://doi.org/10.21161/mjm.180170

[7] Khan, M. A. H. and Hossain, I. (2013) Leaf Spot Disease of Coconut Seedling and Its Eco-Friendly Management. *Journal of Bangladesh Agriculture University*, **11**, 199-208. https://doi.org/10.3329/jbau.v11i2.19894

[8] White, T.J., Bruns, T.D., Lee, S.B. and Taylor, J.W. (1990) Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: Innis, M.A., Gelfand, D.H., White, T.J., *et al.*, Eds., *PCR Protocols: A Guide to Methods and Ap-*

plications, Academic Press, Cambridge, 315-322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

[9] Berbee M., Pirseyedi M. and Hubbard, S. (1999) *Cochliobolus* Phylogenetics and the Origin of Known, Highly Virule Pathogens, Inferred from ITS and Glyceraldehyde-3-Phosphate Dehydrogenase Gene Sequences. *Mycologia*, **91**, 964-977. https://doi.org/10.1080/00275514.1999.12061106

[10] Schoch, C., Crous, P.W., Groenewald, J., Boehm, E., Burgess, T.I., De Gruyter, J., De Hoog, G., Dixon, L., Grube, M., Gueidan, C., *et al.* (2009) A Class-Wide Phylogenetic Assessment of *Dothideomycetes*. *Studies in Mycology*, **64**, 1-15. https://doi.org/10.3114/sim.2009.64.01

[11] Sezer, A. and Dolar, F.S. (2012) *Colletotrichum acutatum*, a New Pathogen of Hazelnut. *Journal of Phytopathology*, **160**, 428-430. https://doi.org/10.1111/j.1439-0434.2012.01910.x

[12] Islam, M.R. (2011) Studies on Seedling Disease of Jujube and Coconut in Jhenaidah and Jessore. M.S. Thesis, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, 1-44.

[13] Manamgoda, D.S., Lei, C., McKenzie, E.H.C., Crous, P.W., Madrid, H., Chukeatirote, E., Shivas, R.G., Tan, Y.P. and Hyde, K.D. (2012) A Phylogenetic and Taxonomic Re-Evaluation of the *Bipolaris* - *Cochliobolus* - *Curvularia* Complex. *Fungal Diversity*, **56**, 131-144. https://doi.org/10.1007/s13225-012-0189-2

[14] Marin-Felix, Y., Senwanna, C., Cheewangkoon, R., Crous, P.W. (2017) New Species and Records of *Bipolaris* and *Curvularia* from Thailand. *Mycosphere*, **8**, 1555-1574. https://doi.org/10.5943/mycosphere/8/9/11

[15] Wang, H., Xu, L., Zhang, Z., Lin, J., Huang, X. (2018) First Report of *Curvularia pseudobrachyspora* Causing Leaf Spots in *Areca catechu* in China. *Plant Disease*, **103**, 150. https://doi.org/10.1094/PDIS-06-18-1096-PDN

[16] Xu, G., Qiu, F., Zhou, J., Zheng, F.Q., Zheng, L., Miao, W.G. and Xie, C.P. (2019) First Report of *Curvularia oryzae* Causing Leaf Spot of Coconut Seedling in China. *Plant Disease*, **104**, 574. https://doi.org/10.1094/PDIS-08-19-1575-PDN

[17] Fernando, P. and Mahindrapala, R. (1977) Leaf Blight Disease of Coconut: 1. Studies on *Pestalotia palmarum* Cooke. *Ceylon Coconut Quarterly*, **28**, 73-80.

[18] Ram, C. (1989) Mycoflora Associated with Leaf Blight of Coconut Palm. *Fitopatologia Brasileira*, **14**, 36-38.

[19] Sugata Ghose (2014) Studies on Coconut Leaf Diseases in Orissa and Their Management Department of Plant Pathology College of Agriculture Orissa University of Agriculture and Technology, Bhubaneswar, 1-90.

[20] Cardoso, G.D., Barreto, A.F., Arauja, E., de Almeida, F.A. and de Carvalho, R.A.G. (2003) Ethiology and Progress of the Pestalotiopsis Leaf Spot in Coconut Plantations at the District of São Gonçalo, Sousa, Paraíba State. *Revista Brasileira de Fraticutura*, **25**, 335-336. https://doi.org/10.1590/S0100-29452003000200039