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IMPORTANCE OF FUNGAL ROOT ROT PATHOGENS OF CASSAVA IN BENIN

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Abstract

Cassava root rots are important diseases in different agroecological zones of West Africa. The causing pathogens were collected from 101 farmers' fields in forest mosaic savanna, southern guinea savanna, northern guinea savanna and sudan savanna of Benin were isolated, identified and analyzed for their virulence. A total of 51 isolates were found showing different disease reaction with 13.7% highly virulent, 17.6%, virulent and 13.7% moderately virulent. The potential root rot pathogens found in the agroecological zones of Benin were *Botryodiplodia theobromae* accounting for 66.7% (most frequently in the southern guinea savanna and the forest mosaic savanna), *Fusarium solani* for 11.7%, *Fusarium oxysporum* for 9.1%, *Nattrasia mangiferae* for 3.9%, *Fusarium semitectum* for 1.9% and *Sclerotium rolfsii* for 1.9%. *Nattrasia mangiferae* was found only in the sudan savanna at the frequency of 3.9%. *Trichoderma* and *Rhizopus* species were also found during our investigations but theirs effects were non virulent on cassava root.

Key words: Benin, Cassava, Soil borne pathogens, Virulence.

Introduction

Cassava (*Manihot esculenta* Crantz) is an important food crop in tropical Africa, Asia and Latin America, with a total world production estimated at 204 million tonnes in 2013 (Anon., 2015). The sub Saharan region of Africa grows 55% of the world production of cassava roots. It is the cheapest known source of starch, and is used in more than 300 industrial products (Anon., 2008). One promising application is the fermentation of the starch to produce ethanol used in biofuel. In Benin, cassava is one of the most cultivated root crops and the first food crop with 4 066 711 tons followed by yam in 2014. The crop is attacked by several diseases among which the root rot is widespread and economically important in tropical Africa. For instance, in Congo losses due to the root rot diseases in cassava have been reported to be up to 30 and 80 percent (Makambila & Koumono, 1994).

Many microorganisms can be responsible for the disease. Hillocks & Wydra (2002) listed the organisms known to cause or to be involved in the root rot complex. In the Democratic Republic of Congo, *Pythium* species, *Fusarium* species, and *Spherostibe repens* occur widely, whereas in Nigeria and Togo, *Botryodiplodia theobromae (Lasiodiplodia theobromae)* was reported as the dominant pathogen closely followed by *Fusarium* species (Makambila, 1994; Boher *et al*., 1997; Onyeka *et al.*, 2005, Banito *et al.*, 2010). In Cameroon, the commonly recovered fungi were *B. theobromae* and *Armillaria* spp., and 30% of the rotted tubers were infected by *Fusarium* spp. (Bandyopadhyay *et al.*, 2006). In the Central region of Ghana the main fungal pathogens found to be associated with the root rot disease are white *Fusarium* and dark *Fusarium* species, with *Sclerotium rolfsii*, *Fusarium solani*, *Fusarium oxysporum*, *Botryodiplodia theobromae* and *Aspergillus* as other potential root rot pathogen in the soil. As important causal pathogens of the root rots in Cameroon, Nigeria and Benin, *B. theobromae*, *Sclerotium rolfsii* and *Fusarium* spp. were identified (Afouda *et al.*, 1995; Afouda & Wydra, 1996). In Benin, *Nattrassia mangiferae* is reported to be the most important pathogen (Msikita *et al.*, 2005), but the investigations were conducted in few localities of Benin.

The aim of this study was to determine the present status of cassava root rot in four agroecological zones of Benin.

Materials and Methods

The study area: The study was carried out in the Republic of Benin (West Africa), situated between the latitudes 6°10' and 12°25' and the longitudes 0°45' and 3°55'. The average annual rainfall fluctuates from 900 mm to 1 300 mm, and the average annual temperature varies from 26°C to 28°C.

The study area covered the four agro-ecological zones of Benin (Fig. 1) (Hell *et al.*, 2000): (1) the forest mosaic savanna (FMS) situated between the latitudes 6°30 and 7° north including the localities of Adjohoun and Lokossa. The FMS is characterised by two rainy seasons from April to July, and September to November, alternating with a long dry season from December to February, and a short dry season from July to august, which rarely exceeds two months. The average relative humidity exceeds 90% during almost all year, and the average annual temperature ranges from 25°C to 28°C and can exceptionally reach 35-40°C, (2) the southern guinea savanna (SGS), from the latitude 7° to 8° North, includes the localities of Glazoué and Savalou. The SGS zone is a transition zone located between the North and the South of Benin, with the same seasonal pattern as the FMS, but less humid than the FMS zone. The average relative humidity ranges from 80% up to 85% during the rainy period of the year, and the maximum temperature more often between 28 and 32°C, (3) the northern guinea

savanna (NGS) situated between the latitudes 8° and 11° North, in contrast, is characterised by one rainy season from April to September. The relative humidity is only high, more than 70%, during a short period running from July to September, very low from November to February, and a high temperature (28 to 35°C). The NGS includes the localities of N'Dali, Parakou, Ouèssè and Savè in the northeast and the centre of the country, (4) the sudansavanna (SS) is comprised between the latitudes 11° and 12° North with one rainy season running from May to September. This area, including the localities of Natitingou, Kouandé and Kandi, is dominated by the Atacora chain and is characterized by the atacorian climate type with a low average relative humidity (less than 60%) during several months, a high temperature (30-42°C) and an annual rainfall up to 1 300 mm.

The study area is essentially dominated by tropical ferruginous soils originated from granite and gneiss (Dubroeucq, 1977).

Site selection and cassava root collection: Cassava roots were collected in December 1999 and in September 2000 from 101 farmers' fields randomly selected in the above mentioned agroecological zones where cassava is cropped. Fields with at least of 150 m square size were selected, and in each field, 20 plants selected on the field diagonals (10 plants on each diagonal) were assessed for rot symptom. The main symptom of rot disease is a breakdown in tissue of the mature tuberous roots, usually associated with a fool odor and changes in color, which may be useful in distinguishing the pathogens involved. Each root rot sample was collected in a journal paper for the pathogen isolation in the laboratory.

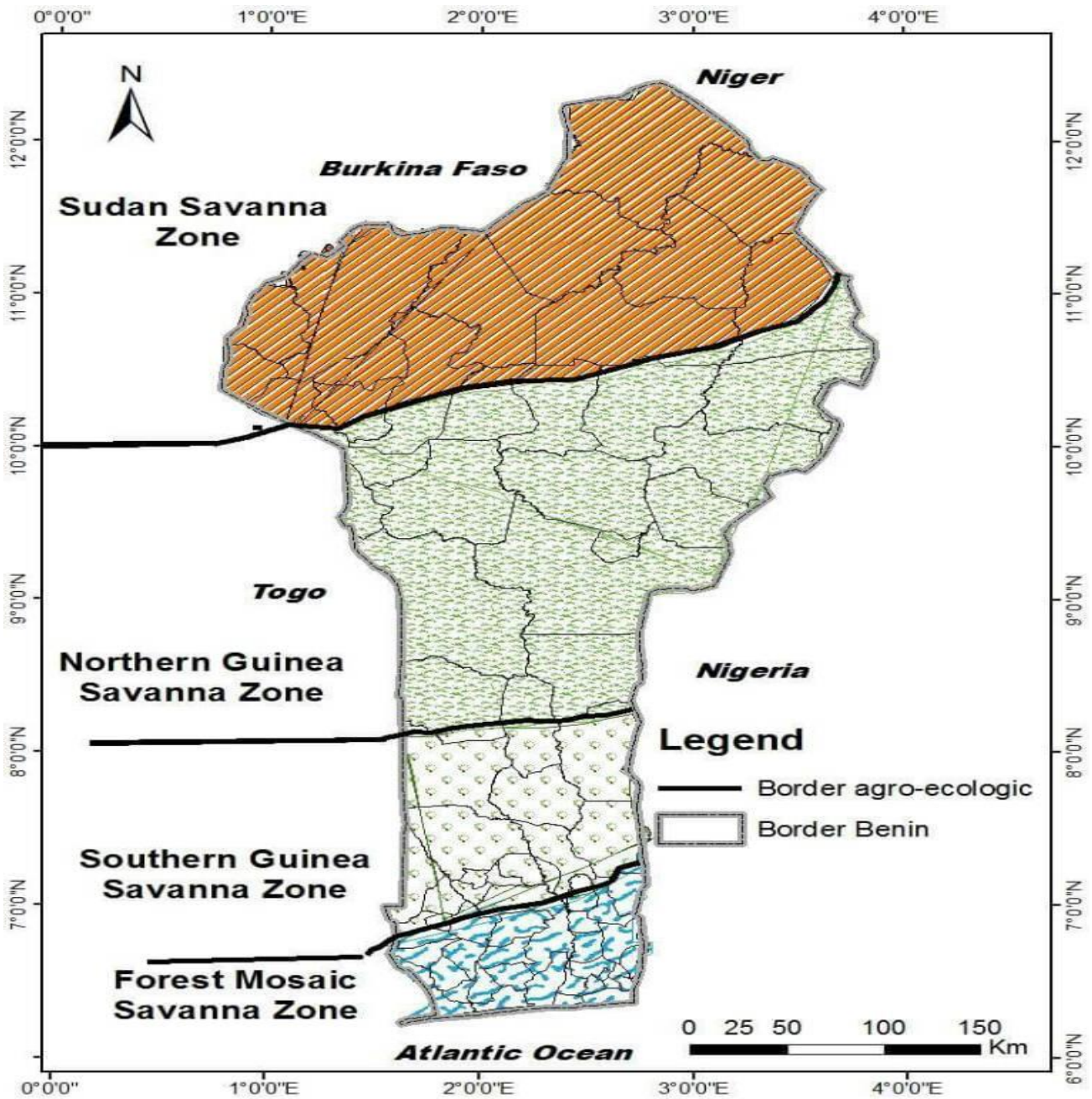

Fig. 1. Map of the Republic of Benin with the different agro-ecological zones.

Root rot identification: Cassava roots with rot symptoms were brought at the laboratory at the International Institute of Tropical Agriculture, Benin Station, for the pathogens identification. They were washed several times in running tap water and then in sterile distilled water. The part between the healthy part and rot part was cut and washed in three changes of sterile distilled water. The pieces were surface disinfected in a solution of 1% of NaOCl for ten minutes and further rinsed in distilled water then blotted dry. Small pieces containing the rots parts and the healthy parts were cut and placed on Potato Dextrose Agar (PDA) containing 5% of streptomycin sulfate for colony characters observation. After incubation, subcultures were done. For microscopic characters, Malt Extract Agar (MEA) and Oat meal Agar (OA) were prepared. The plates were incubated at 25°C for 7-10 days for some structures in the aerial mycelium observation and for 3-4 weeks for chlamydospores. Fungal structures are suspended in cotton blue on a microscope slide and observed under the microscope. Identification of the fungi was done with the aid of appropriate keys by culture appearance and by microscopical observation of fruiting bodies and spores.

Fungi virulence test: Cassava tubers of 8 months old plants of a susceptible local variety Agric were used. Culture of each fungus isolated in a Petri dish on PDA was let grow till 9 days. The tubers were washed in running tap water and disinfested in a solution of 1% NaOCl for ten minutes and rinsed 3 times with tap water. Two holes of 12 mm diameter were bored in each tuberized root and inoculated with a 12 mm diameter circle of medium with fungi cut with a sterile cork borer under a laminar. The holes of each tuber were covered with sterile cotton wetted with sterile water. For incubation tubers are kept in plastic bags in order to maintain humid condition. Water was added in order to maintain wet atmosphere. For each species of fungi, 3 tubers were used for infection. After 4 days, the rotten parts of the roots are cut out. The fresh weight of rotten parts is measured. Dry weight is determined after drying the rot in aluminum paper at 105ºC for 24 h.

Statistical analyses: Root rot dry weights were compared by ANOVA following log (x+1)-transformation. Means were separated by the Student-Newman-Keuls test at $p \leq 0.05$. The isolates were grouped into virulence levels based on the mean percentage scores averaged recorded as follows 0-20% = non virulent; 20.1-40.0% = moderately virulent; 40.1-60.0% = virulent and > 60% = highly virulent (Onyeka, 2002).

Results

From the 101 cassava root samples collected in the different agro-ecological zones, fungi were found only in 51 samples, and no fungi were recovered from the others cassava root samples (Tables 1 & 2). Across the different agro-ecological zones, a set of fungi including *Botryodiplodia theobromae*, *Nattrassia mangifera*, *Sclerotium rolfsii*, *Fusarium oxysporum*, *Fusarium semitectum*, and *Fusarium solani* has been identified. Out of the 51 fungi isolates, 13.7% showed the disease reaction with highly virulence, while 17.6% showed the disease virulence (Table 1). Among the highly virulent fungi, 87.5% accounted for *Botryodiplodia theobromae*, and occurred for 57.1% in the forest mosaic savanna (FMS). The virulent fungi were present in the southern guinea savanna (SGS) at 55.5% with *B. theobromae* the most representative followed by the FMS zone with *B. theobromae* occurring on the cassava tubers at 33.3% (Table 1). Among the highly virulent fungi *Nattrassia mangiferae* occurred in the sudan savanna (SS) while *Fusarium solani* was found in the northern guinea savanna (NGS) (Table 1).

Based on the disease reaction, 68.6% isolates of the observed fungi were less virulent in the fourth agro-ecological zones of Benin among them 54.9% were non virulent (Table 2). Among the less virulent fungi, *B. theobromae* isolates accounted for 57.1% and occurred in the FMS zone (74.2%). The less virulent isolates were *B. theobromae* and *Nattrasia mangiferae* isolates. The non virulent isolates were *Fusarium solani*, *Fusarium oxysporum*, *Fusarium semitectum* and *Sclerotium rolfsii*, while *B. theobromae* isolates could be non virulent and moderately virulent.

The potential root rot pathogens found in the different agroecological zones were *Botryodiplodia theobromae* accounting for 66.7% (most frequently in the SGS and the FMS), *Fusarium solani* for 11.7%, *Fusarium oxysporum* for 9.1%, *Nattrasia mangiferae* for 3.9%, *Fusarium semitectum* for 1.9% and *Sclerotium rolfsii* for 1.9%. *Nattrassia mangiferae* was found only in the NSS.

Trichoderma and *Rhizopus* species (results not shown) were also found during our investigations but their effects were non virulent on cassava root.

Discussion

The most common cassava root rot pathogen isolated was *B. theobromae* mainly in the forest mosaic savana and in southern guinea savanna. This situation could be due to the weather conditions in the two agro-ecological zones, and then the two zones are humid and greatly rainy. It was reported that *B. theobromae* is widespread on harvested stems (Hillocks & Wydra, 2002). The fact that *B. theobromae* have been recovered from cassava root rot is that cassava has a long vegetative cycle (Onyeka *et al.*, 2005), and thus the pathogens have sufficient time to move and infect the root.

Fusarium species were found everywhere without specification to the agro-ecological zone. Both *F. oxysporum* and *F. solani* species complex are genetically diverse and common in soils worldwide.

In Nigeria, *B. theobromae* was found as the most frequently isolated pathogenat 75% frequency,while *F. solani* and *F. oxysporum* together were isolatedat a frequency of 45% (Onyeka, 2002). *Botryodiplodia theobromae* was consistently the most important pathogen in Nigeria (Akinyele & Ikotun, 1989; Onyeka *et al.*, 2004). Isolates of *B. theobromae* were identified in more than 70% of 115 root rot-infected cassava fields sampled across three agro-ecological zones in Nigeria (Onyeka *et al.*, 2004).

Table 1. List of the most virulent fungi identified in the ecozones of Benin.

Isolates	Locations	Rot root dry weight	Fungi	Virulence level
Z_{34}	SGS	181.31 a	*B. theobromae*	Highly virulent
Z_{27}	SGS	164.90 ab	*B. theobromae*	‘’
O_1	FMS	156.99 abcd	*B. theobromae*	‘’
M_{17}	FMS	155.67 abcd	*B. theobromae*	‘’
At_1	FMS	126.82 abcd	*B. theobromae*	‘’
A_1	SS	126.69 abcd	*N. mangiferae*	‘’
M_{11}	FMS	120.65 abcd	*B. theobromae*	‘’
Z_{19}	SGS	108.48 abcd	*B. theobromae*	Virulent
Z_{12}	SGS	108.10 abcd	*B. theobromae*	‘’
B_1	NGS	103.76 abcd	*Fusarium solani*	‘’
O_3	FMS	103.69 abcd	*B. theobromae*	‘’
Z_{29}	SGS	91.77 abcd	*B. theobromae*	‘’
Z_{31}	SGS	90.54 abcd	*B. theobromae*	‘’
Z_{25}	SGS	85.87 abcd	*B. theobromae*	‘’
M_6	FMS	85.71 abcd	*B. theobromae*	‘’
M_{13}	FMS	75.25 abcd	*B. theobromae*	‘’

Letters affected to the isolates are abbreviation of department names: A = Atacora, B = Borgou, M = Mono, O = Ouémé, At = Atlantique, Z = Zou; FMS = Forest Mosaic Savannah; SGS = Southern Guinea Savannah; NGS = Northern Guinea Savannah; SS = Sudan Savannah. Values followed by different letters are significantly different according to Student Newman-Keuls test at $p \leq 0.05$

Table 2. List of the less virulent fungi identified in the different ecozones of Benin.

Isolates	Locations	Rot root dry weight	Fungi	Virulence level
Z_{28}	SGS	68.40 abcd	*Botryodiplodia theobromae*	Lowly virulent
M_8	FMS	61.12 bcd	*B. theobromae*	‘’
A_2	SS	56.55 bcd	*Nattrassia mangiferae*	‘’
M_3	FMS	53.67 bcd	*B. theobromae*	‘’
Z_1	SGS	51.23 bcd	*B. theobromae*	‘’
M_{16}	FMS	43.00 cd	*B. theobromae*	‘’
M_{19}	FMS	39.89 cd	*B. theobromae*	‘’
O_2	FMS	35.58 cd	*Sclerotium rolfsii*	Very lowly virulent
Z_4	SGS	32.71 d	*B.theobromae*	‘’
M_{21}	FMS	31.56 d	*B. theobromae*	‘’
Z_{32}	SGS	30.97d	*B. theobromae*	‘’
M_{23}	FMS	30.45 d	*B. theobromae*	‘’
At_2	SS	30.38 d	*Fusariumoxysporum*	‘’
M_9	FMS	29.67 d	*B. theobromae*	‘’
Z9	SGS	25.45 d	*B. theobromae*	‘’
M_{22}	FMS	22.93 d	*B. theobromae*	‘’
Z_6	SGS	22.07 d	*B. theobromae*	‘’
M_{14}	FMS	17.87 d	*B. theobromae*	‘’
Z_{17}	SGS	15.94 d	*B. theobromae*	‘’
B_5	NGS	11.64 d	*F. oxysporum*	‘’
A_7	SS	9.85 d	*F. semitectum*	‘’
Z_{10}	SGS	7.62 d	*F. solani*	‘’
A_6	SS	7.52 d	*F. oxysporum*	‘’
Z_2	SGS	6.25 d	*F. oxysporum*	‘’
Z_{20}	SGS	5.69 d	*B. theobromae*	‘’
Z_{40}	SGS	4.87 d	*B. theobromae*	‘’
M_{12}	FMS	4.80 d	*F. oxysporum*	‘’
Z_{13}	SGS	4.26 d	*B. theobromae*	‘’
Z_{33}	SGS	3.69 d	*F. solani*	‘’
M_5	FMS	3.34 d	*F. solani*	‘’
M_{15}	FMS	3.29 d	*F. solani*	‘’
Z_{16}	SGS	3.16 d	*B. theobromae*	‘’
Z_3	SGS	2.96 d	*F. solani*	‘’
M_4	FMS	2.25 d	*F. solani.*	‘’
M_{10}	FMS	2.15 d	*F. solani*	‘’

Letters affected to the isolates are abbreviation of department names: A = Atacora, B = Borgou, M = Mono, O = Ouémé, At = Atlantique, Z = Zou; FMS = Forest Mosaic Savannah; SGS = Southern Guinea Savannah; NGS = Northern Guinea Savannah; SS = Sudan Savannah. Values followed by different letters are significantly different according to Student Newman-Keuls test at $p \leq 0.05$.

As important causal pathogens of root rots in Cameroon, Nigeria and Benin, *B. theobromae*, *Sclerotium rolfsii* and *Fusarium* spp. were also identified (Afouda *et al.*, 1995; Afouda & Wydra, 1996; Bandyopadhyay *et al.*, 2006). Msikita *et al.* (1997) in a survey conducted duringthe dry season in 1996, isolated *B. theobromae* at a frequencyof 28% in Nigeria and 7.7% in Benin, while *Fusarium* species were isolated at a frequency of 13 and 12% in Nigeria andBenin respectively.

Our results were not similar to those found by Msikita *et al.* (2005) which stated that *N. mangiferae* was the most frequently isolated pathogen during the dry season in Nigeria and Benin. We only found *N. mangiferae* in the Northern Guinean Savanna at the frequency of 3.9%. In Nigeria, Onyeka (2002) did not report the presence of *N. mangiferae.* However, Bandyopadhyay *et al.*, 2006 stated that *N. mangiferae* is adapted to diverse ecological conditions and that it can thrive well even in areas with <1,000 mm of rainfall per year, whereas *B. theobromae*, *F. solani*, and *F. oxysporum* are more frequent in areas with >1,600 mm of rainfall per year.

The different fungi isolates associated with cassava root rot disease varied significantly in aggressiveness. Various fungi species, *Pythium*, *Spherostibe repens* and *Armillaria* spp., with different virulence level were found under different environment (Makambila, 1994; Boher *et al.*, 1997; Bandyopadhyay *et al.*, 2006). The different level in virulence noticed in our study could be due to the susceptibility of the cassava genotypes.

Trichoderma, *Aspergillus* and *Rhizopus* species were recorded, but their effects were non virulent on cassava root, so they could be secondary pathogens after primary pathogen infection.

It is known that *Aspergillus* and *Fusarium* species could produce mycotoxins. In Benin, cassava chips are consumed and could be a serious risk to consumer health when mycotoxin's contamination occurred. Gnonlonfin *et al.* (2008) found *Aspergillus flavus* as the predominant fungal species on cassava chips during two consecutive seasons in two agroecological zones of Benin (northern guinea savanna and sudan savanna) but the high performance liquid chromatography analysis did not showed contamination by either aflatoxin or fumonisin. In contrast, Manjula *et al.* (2009) studied the occurrence of *Aspergillus flavus*, *Fusarium* spp. and related fungi, and resultant aflatoxins and the related mycotixins in dried cassava samples from various markets and villages in Tanzania and Congo, and found aflatoxin and fumonisin in cassava chips and flour.

Several authors distinguished different genera of cassava root rot pathogens and which can be divided into three groups based on the symptoms produced: (i) soft root rot where the main species involved are *Phytophthora* spp. and *Pythium* spp.; (ii) dry root rot, caused by *Fusarium* spp., *Corallomycetella repens* (equivalent to *Nectria mauritiicola* and *Sphaerostilbe repens*), *Armillaria mellea* and *Sclerotium rolfsii*; and (iii) black root rot, caused by Botryosphaeriaceae species such as *Neoscytalidium hyalinum*, *Lasiodiplodia* spp. and *Nattrassia mangiferae* (Bandyopadhyay *et al*., 2006; Okechukwu *et al*., 2009; Banito *et al*., 2010; Guo *et al*., 2012; Machado *et al*., 2014; Vilas Boas *et al*., 2017). Also, Oliveira *et al*. (2017) reported that distribution of the root rot symptoms caused by *Fusarium* spp, *Phytophthora* spp. and Botryosphaeriaceae species indicates the presence of quantitative inheritance. Controls of some of these pathogens on other host plants were observed (Malik *et al*., 2015; Akhtar *et al*., 2017; Ullah *et al*., 2017). But, integrated disease management based on planting resistant cultivars associated with soil and water management practices, crop rotation and use of healthy, high-quality planting material are reliable techniques to control cassava root rot.

Conclusion

From the research outcomes, it appears that four fungi species were found to be associated with cassava culture in Benin with different virulence level. *Botryodiplodia theobromae* is an important component of this complex, and there is no conclusive evidence that crop variety influences the constituents of this fungi complex. Further research works need to be conducted to better understand the role and the ability of each the fungi involved in the colonization of cassava root and the interaction between isolates and genotypes for a sustainable cassava crop management.

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