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PHYSIOLOGICAL STUDIES OF FUNGI COMPLEXES ASSOCIATED WITH CASHEW DISEASES

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ABSTRACT

A field study to determine the incidence of fungi complexes associated with cashew diseases revealed three fungi of genera *Lasiodiplodia*, *Fusarium* and *Pestalotia* when cultured on Potato Dextrose Agar medium. The optimum temperature for the growth of *Fusarium* was 25°C while all the temperature treatments 20°C, 25°C and 30°C were found suitable for the growth of *Lasiodiplodia* and *Pestalotia*. It was also found that alternating 12 hours light plus 12 hours darkness was more suitable for the growth of *Fusarium* than continuous darkness. The growth of both *Lasiodiplodia* and *Pestalotia* were not affected by the light condition.

Keywords: fungi complexes, cashew, *fusarium*, growth medium, *lasiodiplodia*, light, *pestalotia*, temperature.

INTRODUCTION

Cashew (*Anacardium occidentale*) is next to cocoa as export crop and a major source of cash income to many small holder farmers in the Central and Northern parts of Nigeria (Topper *et al.*, 2001; CBN, 2005). Cashew provides considerable contributions to Gross Domestic product, National income and generation of foreign exchange like cocoa, palm produce, cotton and sesame (Aliyu and Hammed, 2008). Cashew in the 15th century was used mainly for afforestation scheme for the control of erosions in Nigeria, however the commercial plantations started in the early 1950. The introduction of Brazilian cashew biotype with improved and desirable nut and kernel quality characteristic of Cocoa Research Institute of Nigeria further increased the spread of the crop and popularity in Nigeria (Hammed *et al.*, 2007).

Nigeria is the second world producer of cashew nut after Vietnam and has seven diverse agro - ecologies; however cashew can only be economically cultivated in woodland - tall - grass savanna and rainforest ecologies. The crop able lands spread across 27 states of Nigeria and the producing states are grouped into major and minor on the basis of current production levels (Aliyu and Hammed 2008).

Cashew being a crop of considerable economic importance in Africa, Asia and Latin America is being affected by diverse diseases. Inflorescence dieback is a serious disease of cashew caused by *Lasiodiplodia theobromae*. This reduces the fruit bearing of cashew in Nigeria. Over 50 disease pathogens and agents that constitute pests on cashew throughout the world were recorded (Teixeira, 1988). These pathogens are reported to be site-specific (Grundon, 2000). Twig dieback caused by *Lasiodiplodia theobromae* has remain a major factor limiting cashew production for decades in Nigeria especially on young cashew plot (Hammed and Adedeji, 2008).

Bearing in mind the economic importance of cashew and the disease complexes associated with the crop, this study which was aimed at isolating and identifying the pathogens associated with inflorescences

and twig of cashew will give a better understanding of the organisms involved in the disease expression and serves as a pointer towards control mechanisms.

METHODOLOGY

Disease sample collection

A survey of cashew growing ecologies in South West and North Central of Nigeria was carried out to reveal the symptoms and severity of cashew diseases. The naturally diseased cashew showing the typical symptoms of diseases were selected from the plots and farmers' field. Samples of diseased inflorescences, twig and leaves were collected for the isolation of associated pathogens.

Isolation of pathogens

Isolation of pathogens from samples was carried out on potato dextrose agar (PDA) medium. Sections of 3 - 5 mm diameter were cut using sterilized scalpel from the periphery of the lesions on leaves and twigs while the whole infected inflorescence were sectioned. Pieces of diseased tissues were surface sterilized in 2% sodium hypochlorite solution for 3 minutes and washed in three changes of sterile distilled water. Sterilized pieces were blotted dry in between sterile filter paper and inoculated on PDA acidified with 10% lactic acid in Petri dishes. Petri dishes were incubated at 25±2°C. Colony growth was observed daily for 7 days depending on when growth occurred. Cultures were hyphal - tip - transferred to obtain pure cultures. Identification of cultures were done by microscopic examination and compared with reference cultures.

Pathogenicity

To test for the pathogenicity of the isolates, healthy branches, inflorescence and leaves on cashew plants were selected. A sterilized 6 mm disc borer was used to create wound on the healthy branches and leaves and inoculated with 6 mm disc of culture isolates from naturally infected parts in the field. A wound was made on and inoculated with 6 mm disc of only PDA to serve as



control and all wrapped with parafilm. These were replicated six times. For the healthy inflorescences, spore suspensions of the culture isolates were sprayed on them and the unsprayed served as control. The cashew parts were observed for the development of disease symptoms.

Effect of different temperature on mycelial extension of pathogens

Six millimeter culture discs were cut with sterilized cork borer from advancing margin of colonies of isolates and incubated on PDA at 20°C, 25°C and 30°C. This experiment was replicated in triplicates and mycelial growth was recorded after seven days. The experiment was conducted in completely randomized design (CRB) and the data were statistically analyzed (Steel and Torrie, 1986).

Effect of light and darkness on mycelial growth of isolates

For the effect of light and darkness on mycelial growth of isolated fungi, 6 mm culture discs were cut with sterilized cork borer from advancing margins of the

colonies of isolated fungi and inoculated on PDA plates separately for seven days. Carbon paper was used to wrap the petri dishes for darkness. All petri dishes were incubated at 25±2°C in triplicates in alternating light and darkness (12 hours light plus 12 hours darkness) and complete darkness (24 hours).

RESULTS

Isolation of pathogen and pathogenicity

Fungi complexes associated with cashew leaf, twig and inflorescence diseases isolated revealed three fungi of genera *Lasiodiplodia*, *Fusarium* and *Pestalotia* species. *Lasiodiplodia* and *Fusarium* species were isolated from diseased inflorescences, *Lasiodiplodia* and *Pestalotia* species from infected twigs while *Fusarium* and *Pestalotia* species were associated with leaf blight of cashew (Table-1). The study was limited to diseases that affect cashew leaves, twigs and cashew inflorescences. The pathogens were re-isolated from the disease symptoms expressed from the inoculated branches and leaves and sprayed inflorescences after seven days of incubation.

Table-1. Occurrence of organisms associated with cashew.

Plant parts	Associated organisms		
	<i>Lasiodiplodia</i> spp	<i>Fusarium</i> spp	<i>Pestalotia</i> spp
Inflorescences	+	+	-
Twigs	+	-	+
Leaves	-	+	+

Present (+), Absent (-).

Effect of light and darkness on mycelial growth

The condition of light was revealed to have effect on the growth and mycelial extension of the associated organism as shown in Table-2. *Lasiodiplodia* and *Pestalotia* species growth were not affected by the

condition of light and darkness as both extended to the capacity of the petri plates, while *Fusarium* spp was seen to have a slow growth rate with 61.0 mm and 33.5 mm in diameter at the condition of alternating light and darkness and continuous darkness, respectively.

Table-2. Effect of light conditions on the growth of the isolates.

Light conditions	Mean mycelial growth (mm) of the isolates		
	<i>Lasiodiplodia</i> spp	<i>Fusarium</i> spp	<i>Pestalotia</i> spp
Alternating light + darkness	85.0a	61.0a	85.0a
Continuous darkness	85.0a	33.5b	85.0a

Each value is the mean of 3 replicates. Means followed by the same letter in the same column are not significantly different according to LSD (5%)

Effect of temperature on mycelial extension

Table-3 shows the effect of different temperature on the mycelial growth of the fungi. *Fusarium* spp had its

best growth at 25°C with 61.0 mm diameter, which differ significantly with that of 20°C and 30°C while *Lasiodiplodia* and *Pestalotia* species had 85.0mm growth rate each in all the temperature conditions.

**Table-3.** Effect of temperature on growth of the isolates.

Temperature ($^{\circ}$ C)	Mean mycelial growth (mm) of the isolates		
	<i>Lasiodiplodia</i> spp	<i>Fusarium</i> spp	<i>Pestalotia</i> spp
20	85.0a	34.25c	85.0a
25	85.0a	61.0a	85.0a
30	85.0a	41.0b	85.0a

Each value is the mean of 3 replicates. Means followed by the same letter in the same column are not significantly different according to LSD (5%)

DISCUSSIONS

The fungi complexes: *Lasiodiplodia*, *Fusarium* and *Pestalotia* species associated with cashew diseases were isolated from the inflorescences, twigs and leaves of the plant. It was interesting to note the occurrence of the fungi in more than one of the targeted cashew parts. *Lasiodiplodia* species occurred in the inflorescence and twigs, *Fusarium* species was found in the inflorescence and leaves while *Pestalotia* species was isolated from the twigs and leaves. The isolation of the pathogens from the artificially inoculated and sprayed branches, leaves and inflorescences, respectively confirm pathogenicity according to Koch's postulates.

The pathogenicity of *Lasiodiplodia theobromae* as the causal organism of inflorescence and twig dieback of cashew has been reported by earlier workers (Olunloyo, 1983; Hammed and Adedeji, 2008). *Lasiodiplodia theobromae* occurrences have also been established as pathogen in banana fruit (Mortuza and Ilag, 1999), gummosis in *Jatropha podagrica* (Fu *et al.*, 2007), mango dieback (Narasimhudu and Reddy, 1992; Khanzada *et al.*, 2004a; 2004b; Al Adawi *et al.*, 2003; Savant and Raut, 2000). *Lasiodiplodia theobromae* has also been found in diseased situation of some other crops: leaf necrosis and stem cankers on *Protea magnifica* (Denman *et al.*, 2003), gummosis disease of Japanese apricot and peach trees (Li *et al.*, 1995), canker in white cedar (Sand rock *et al.*, 1999), fruit rot of coconut, soft rot of pawpaw, guava, litchi, sapodilla fruit and dieback in lemon plant fruits (Anthony *et al.*, 2004; Alam *et al.*, 2001; Mortuza and Ilag 1999).

Also, *Fusarium* species as been reported as pathogenic on some crops; rot of stored yam tuber (Ogundana *et al.*, 1970) spot diseases of some ornamental plants (Suleiman and Odebode, 2003) root rot and wilt of coffee (Hill and Waller, 1990) and wilt of *Solanum melogena* (Siva *et al.*, 2008) among others. *Fusarium* survive a wide range of environmental condition as conidia on plant debris in the soil was the reason for its wide tropical sub tropical and warm temperate biogeography distribution. Incidence of *Pestalotia* species on crops was scarce but its occurrence on leaf of cashew was earlier reported by Olunloyo (1975), thus its association in the twig of cashew is of interest.

The co-association of *Lasiodiplodia* and *Fusarium* species on the inflorescence of cashew is not the

first of such existence as it was reported in kolanut (Agbeniyi, 2004) and in Sesham (*Dalbergia sisso* Roxb) (Kausar *et al.*, 2009). There is the need to have a morphological and molecular characterization of these organisms because of their co - association and their wide occurrence on many crops across the tropics and temperate regions.

The effect of light and darkness on mycelial growth of *Lasiodiplodia* species and *Pestalotia* species was not different in this experiment. Both condition of light was found to be suitable for maximum growth of both fungi when the fungi species were exposed to different conditions for seven days. But colony diameter of *Fusarium* spp differs significantly as 33.5mm in continuous darkness to 61.0mm in alternating light and darkness.

The indifference in mycelial growth of *Lasiodiplodia* species in different light conditions contradict the findings of Kausar *et al.*, (2009) on *L. theobromae* isolated from shesam, thus another reason for the morphological and molecular characterization of *Lasiodiplodia* species associated with disease conditions of crops.

Though the mycelial growth of *Lasiodiplodia* and *Pestalotia* species were not affected by conditions of light and darkness but obvious pigmentation of their mycelial after seven days show a difference in their appearance as the *Lasiodiplodia* species under continuous darkness was turning grayish black but remained whitish under alternating light and darkness. Also, *Pestalotia* spp have brown pigmentation radiating from the point of inoculation in the continuous darkness and that of alternating light and darkness remained whitish. The impact of temperature on the growth of different fungi varies. Contrary to the earlier works of Alam *et al.*, (2001) and Kausar *et al.*, (2009) *L. theobroame* have the same mycelial extension rate of 85.0mm diameter in all the temperature conditions. The contradictions raise the question of diversity of organisms (even in the same genera) from crops to crops and locations to locations.

CONCLUSIONS

These findings, therefore, create bases for the morphological and molecular characterization of these organisms for better understanding of their biology, identification and classification.



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