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Isolation and identification of Phytopathogenic fungi responsible for Kolanuts (*Kola acuminate*) rot in Jimeta modern market, Yola Adamawa state Nigeria

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Abstract
This study was aimed at isolation and identification of fungal pathogens responsible for kolanut *C. acuminata* and *C. nitida* (Vent.) rots. Samples of infected kolanut fruits were collected from whole sales stores of the fruit at Jimeta modern market, and conveyed to the labaratory of Biologicl Sciences Department of Modibbo Adama University of Technology, Yola Adamawa State, Nigeria. Potato Dextrose Agar was used as growth medium for the fungi throughout the study. Under aseptic condition different kinds of rot were observed, isolated, identified and proven to be pathogenic through pathogenecity test. *Aspergillus parasiticus*, *Colletotrichum appressoria*, *Colletotrichum cladosporium* and *Cladosporium gloeosporioides Cola acuminata* and *Cola nitida* (Vent.) Were identified with the following incidence; 39.39%, 27.27%, 27.27% and 9.09% respectively. There was a significant difference in disease severity between *C. gloeosporioides* 27.57 and *C. cladosporium*13.37 at P= 0.05 but no significant difference in the means severity of *A. parasiticus and C. appressoria.* The identified fungi were proven to be pathogenic and responsible for the various kinds of rots observed.

Keywords: kola nuts, phytopathogenic fungi, rots and Jimeta modern market

1. Introduction
The Cola species are evergreen, mostly small or moderately sized trees although a few grow to 25 meters. The most commonly used are *C. verticillata* (Thonn.) Starf, *C. acuminata* and *C. nitida* (Vent.) Schott ad Endlicher with the latter two having the greatest economic importance (Lovejoy, 1980) [10]. In the forest areas of West Africa, kola is perhaps second only to palm oil in importance in the list of indigenous cash crops, about 40 Cola species have been described in West Africa (Russel, 1955) [15]. However, in Nigeria, the Cola species of real importance are *C. acuminata and C. nitida* (vent) Schott and Endlicher (Asogwa *et al*., 2006) [5]. Kola is an important economic cash crop to a significant proportion of Nigerian population who are involved in kola farming, trading and industrial utilization (Atanda *et al.,* 2011) [6]. The cultivation of kola began in 19th century and it is estimated that the country produces about 88% of the world's kola nuts with an annual production of 200,000 metric tones mostly from South-western Nigeria (Asogwu *et al*, 2006) [5]. Both species of *C. acuminata and C. nitida* are important economic crops in the forest area of West Africa, Caribbean Island, Mauritius, Sri Lanka and Malaysia (Ndagi *et al*., 2012) [11].
Cola nitida was originally distributed along the west coast of Africa from Sierra Leone to the Republic of Benin with the highest frequency and variability occurring in the forest areas of Côte d'Ivoire and Ghana (Opeke, 1992) [13]. Opeke, 1992 [13] stated that cultivation of C. nitida was carried eastwards through Nigeria towards Cameroon and the Congo around 1900, and spread westwards as far as Senegal (Opeke, 1992) [13]. *C. nitida* is planted through Senegal, Guinea, Liberia, Côte d'Ivoire, and Ghana towards the western part of Nigeria (Voelcker, 1935) [19]. Southern Nigeria is considered the center of occurrence of *C. acuminata*, with its original area of distribution stretching from Nigeria to Gabon. *C. acuminata* also occurred spontaneously in the mountainous areas of Angola, Zaire and Cameroon, and it has long been in cultivation on the islands of Principe and São Tomé (Opeke, 1992) [13]. It has also been cultivated in Angola, Fernando Po and Tanzania. (Fereday *et al*. 1997) [8, 9]. West Africans took the seed with them when they traveled, even in the days of the Slave Trade. Kola has therefore been extensively cultivated in tropical South and Central America and the West Indies.

It has also spread eastwards to Mauritius and Malaysia (FAO, 1982; Purseglove, 1968; Russell, 1955) [7, 14, 15].

The diseases of the stored nuts are the dry rot, grey mould and black rot, their causal agents are *Fusarium solani*, *Botrytis sp* and *Botryodiplodia theobromae*. Grey mould is a serious disease of stored kola nuts, which spread rapidly from nut to nut, the kola nut are covered with lesions having grayish and powdery mycelia. The black rot is characterized by brownish black encrustations which appear in form of spots over black and hard. Also among fungal pathogens of kola nuts *Botryodiplodia theobromae, Fusarium pallidoroseum, Aspergillus* sp, *Penicillium* sp, *Curvularia* sp, and *Mucor* sp. (Agbeniyi *et al*., 2000) [3]. Many fungi are capable of infecting kola fruits at an early stage of development, but the disease symptoms will only develop when conditions are favorable. there is need for identifying the fungi responsible for the rots of the fruits.

The objectives of this research is to Identify the fungal pathogens esponsible for detoriaration of kolanuts in the market

Material and methods

Plating and isolation

Diseased samples of kola nut fruits, *C. acuminata* and *C. nitida* that has all kinds of rots such as dry and internal rots were purchased from the wholesalers of kola nuts in Jimeta modern market. The samples were conveyed to laboratory in a sterile polythene bags for inoculation. A portion of the diseased samples were cut into smaller pieces (2mm) using sterile razor blade for plating in sterile Petri-dishes using sterile spatula (Thomas. 1979) [18]. The pieces of the diseased samples was surfaced sterilized with 0.01% mercuric chloride for 30 seconds, which were rinsed in five different distilled water, and then blotted to dry using filter papers to avoid surface contaminant. The pieces were plated aseptically on 9cm Petri-dishes containing solidified potato dextrose agar (PDA). Solidified plates were inoculated at room temperatures 3-4 days. Fungal colonies growing from the plates were sub-cultured into fresh and sterile media until axenic cultures are obtained (Suleiman and Michael 2013) [17].

Identification of isolates

Microscopic examination was carried out to observe the structure and characteristic of the fungal isolates. A sterile needle was used to pick a little portion of the hyphae containing spores and placed on a sterile glass slide stained with lacto phenol cotton blue and examined under the photographic microscope x40 power objective (Suleiman and Michael 2013) [17]. The morphological and cultural characteristics observed were then compared with the structures in Snowdon (1990) [16].

Pathogenicity Test

Fresh kola nut fruits were obtained and surface sterilized with 0.1% $HgCl_2$ for 1 minute and washed in five changes of sterile distilled water, a sterile dissecting needle was used to puncture and inject fresh healthy kola fruits with 0.3ml spores suspension of the inoculum, using 2.0ml syringe each in three replicates, and kept at room temperature for 1 week to observe whether the isolates are the original cause of the rots to the kola fruit. Similar set up was placed as control using distilled water. The set up was arranged in a complete randomized block design.

Fungal Isolates

The following fungi were isolated, identified and confirmed via pathogenicity test to be pathogens of kola nut fruit in Jimeta Modern Market. *Aspergillus parasiticus* Spear, *Colletotrichum appressoria* (Hutchison) MAFF, *Cladosporium cladosporioides* (Fresen.) G. D Vries. and *Colletotrichum gloeosporioides*.

Growth of pathogens on PDA incubated at 29± 2 ºC

Analysis of variance for colony growth of the pathogens at *P*=0.05 showed significant difference (Table 1). There was, however, no significant difference between A. parasiticus and *C. appressoria* on the first day, *C. gloeosporioides* had the highest diameter on day 1 with (17.77mm), followed by *C. appressoria* while the least was *C. cladosporioides* with (7.87mm).

Results and Discussion

There were four different fungi found associated with kola diseases as oppose to who said kola nuts were robust and have no important diseases. The identified pathogens include *(A. parasiticus, C. appressoria, C. cladosporioides and C. gloeosporioides*) contrary to the findings of (Agbeniyi, 2010) [4] which may be due to differences in climatic factors of the study areas. This is because, control of moisture and temperature levels have prevented mould growth and mycotoxin production Adebajo (1992) [1]. Each of the isolates were proved to be pathogenic with *C. gloeosporioides and A. parasiticus* being the most pathogenic followed by *C. appressoria and C. cladosporioides.* (Adebajo and Papoola 2003) [2] reported *A. Parasiticus* and *Cladosporium* spp *to* be potential pathogens of kola nuts. (Agbeneyi *et al* 2000) [3] reported *Aspergillus* species to be among kola pathogens. There were no literature seen on *Colletotrichum spp*, but generally kola nuts were reported to be susceptible to a wide range of pathogens.

Table 1: Colony Diameter (mm) of Fungal Pathogens of Kola nut on PDA Incubated at 29± 2 ºC for 5 days.

Incubation Period (Days)					
Fungal species	**1**	**2**	**3**	**4**	**5**
*A. parasiticus*14.97	29.80	47.03	69.87	84.67	
*C.apperessoria*15.03	19.87	37.47	43.67	66.77	
C. cladosporioides 7.87	15.00	37.80	44.93	64.93	
C. gloeosporioides 17.77	24.83	39.67	67.80	89.67	
Mean13.91	22.38	40.49	56.57	76.51	
LSD	0.40	0.49	0.73	0.42	0.45

A. parasiticus growth was faster on the second with (29.80mm) followed by *C. gloeosporioides* (24.83mm), *C. appressoria* was (19.87mm) and the least was *C. cladosporioides with* diameter of (15.00mm). On third day

there was no significant difference between *C. appressoria* and *C. cladosporioides*. *A. parasiticus* had the highest diameter on day 3 with growth diameter (47.3mm) followed by *C. gloeosporioides* having (39.67mm). There was also significant difference (*P*=0.05) between *C. appressoria* and *C. cladosporioides* with growth diameters of 43.67mm and 44.93mm respectively A. parasiticus was higher (69.87mm) followed by *C. gloeosporioides* (67.80mm). On last day, *C. gloeosporioides* had the highest growth diameter of 89.67mm and 84.67mm, 66.77mm and 64.93mm of *A. parasiticus*, *C. appressoria* and *C. cladosporioides* respectivel

Table 2: Incidence of Pathogens Isolated from Kola nuts in Jimeta Modern Market

Isolates	Incidence (%)
A. parasiticus	36.36
C. appressoria	27.27
C. cladosporioides	27.27
C. gloeosporioides	9.09
Total	100

Incidence of fungal pathogens

The four different fungal pathogens appeared with varying frequencies which vary significantly at *P* = 0.05, with *A. parasiticus* showing highest incidence of 36.36% followed by *C. appressoria* and *C. cladosporioides* both having 27.27% and least *C. gloeosporioides* 9.09% (Table 2).

Table 3: Diameter of Rot (mm) of Pathogens on Kola

Fungal species	***Means***
A. parasiticus 15.67	
C. appressoria 16.63	
C. cladosporioides 20.37	
C. gloeosporioides 13.57	
LSD	1.22

Severity of pathogens on kola nut fruits

The severity of the fungal isolates responsible for kola nut rots varied significantly at *P* = 0.05 (Table 3) with *C. cladosporioides* having the highest mean diameter rot (20.37mm) and *C. gloeosporioides* having the least (13.57). However, there was no significant difference between the means of *A. parasiticus* and *C. appressoria*.

Conclusion

Four fungi isolated, identified and proved to be pathogenic in *this study involved A. parasiticus, C. appressoria, C. cladosporioides* and *C. gloeosporioides* with *A. parasiticus* had the highest incidence and *C. gloeosporioides the least. C. gloeosporioides* was found to be the most pathogenic among all the pathogens.

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