WebLog Journal of Nutrition and Food Sciences

Research Article
Published: 13 May, 2026

Isolation of Disease Causing Organisms of Kolanut and Types of Kola Buds

Okigbo R. N[1], Ezebo R. O[2]*, Nonyelu I. A[1], Achonye C. C[2] and Mbadugha K. C[3]

[1]Department of Botany, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

[2]Department of Science Laboratory Technology, Anambra State Polytechnic, P.M.B, 002, Mgbakwu, Anambra State, Nigeria

[3]Department of Science Laboratory Technology, Federal College of Land Resources Technology, Obosima/Oforola, P.M.B. 1518, Owerri, Imo State. Nigeria

WebLog Open Access Publications
Article ID : wjnfs.2026.e1301
Author : Ezebo R. O

Abstract

Isolation and identification of fungi pathogens causing kolanut spoilage across Markets in Awka was carried out using standard laboratory procedures. Randomized block sampling method was used in sample collection for the study. Potato dextrose agar was used to prepare innocula for the diseased kolanuts collected. The results of the study revealed the presence of *Aternaria alternata, Fusarium oxysporum, Aspergillus niger* and *Aspergillus flavus*. *Aspergillus niger* was absent only in kolanut samples from Amansea with a percentage occurrence of 75%. *Aspergillus flavus* was present in kolanut samples from all the Markets except Nkwo Amaenyi with percentage occurrence of 75%. *Alternaria sp.* was seen in kolanut samples from all locations with 100% occurrence while *Fusarium oxysporum* was seen in samples of kolanut species collected from Nkwo Amaenyi and Amansea respectively with percentage occurrence of 50%. The results showed that all the fungi pathogens had progressive but slow increase in pathogenicity from day 4 to the 7th day. Although, *A. flavus* had higher pathogenicity on the fresh kolanut samples when compared with other fungi isolates. The study indicated the disease-causing organisms of kolanut across Awka Markets and prescribed ways of identifying and classifying them.

Keywords: Isolation; Disease; Organisms; Kolanut; Types; Kola; Buds

OPEN ACCESS

Correspondence:
Ezebo R. O, Department of Science Laboratory Technology, Anambra State Polytechnic, P.M.B, 002, Mgbakwu, Anambra State, Nigeria,
E-mail: esau_056@yahoo.com

Received Date: *28 Apr 2026*
Accepted Date: *11 May 2026*
Published Date: *13 May 2026*

Citation:
Okigbo RN, Ezebo RO, Nonyelu IA, Achonye CC, Mbadugha KC. Isolation of Disease Causing Organisms of Kolanut and Types of Kola Buds. WebLog J Nutr Food Sci. wjnfs.2026.e1301. https://doi.org/10.5281/zenodo.20210347

Introduction

Kolanut, scientifically known as *Cola acuminata* and *Cola nitida* are evergreen, mostly small or moderately sized trees. Although, a few grow to 25 metres are gotten from *Cola* species. The most commonly used are *C. verticillata, C. acuminata* and *C. nitida*. The species of *Cola* having the greatest economic importance are *Cola acuminata and Cola nitida* [16]. 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 [28]. However, in Nigeria, the *Cola* species of real importance are *C. acuminata* and *C. nitida* [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 [6]. The cultivation of kola began in 19th century and it is estimated that Nigeria produces about 88% of the world's kola nuts with an annual production of 200,000 metric tons mostly from South Western Nigeria [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 [20]. *Cola acuminata* and *Cola nitida*, hold immense cultural, social, and economic significance in many regions of Africa. These nuts are traditionally used in various social and religious ceremonies and are known for their stimulant properties. Traditionally, it has been used as a symbol of hospitality and a key ingredient in various ceremonies and rituals in Nigeria, especially in South Eastern region.

Beyond its cultural importance, *C. acuminata* is frequently used for social and religious ceremonies in Southern and Middle-belt Nigeria while *C. nitida* which is referred to as "the true kola of commerce" has featured in the internal trade of West Africa for a number of centuries (Jaiyeola, 2001). The crop is important because of its nut that has important pharmacological properties [7] and also contains some active principles found in coffee and cocoa (caffeine, theobromine, kolatin) [24] which prevents sleep, thirst and hunger and also acts as an anti-depressant [19]. The presence of alkaloids and other phytochemicals make kolanut suitable for the manufacture of pharmaceutical

products and beverages. The tannin content is also a good source of dyes in textiles and thread [26]. It also has industrial usage for the production of drugs, soft drinks, wines, candies, beverages, animal feed formulation, liquid soap and dyes. However, the quality and safety of kolanuts can be compromised by the presence of fungi. Fungi, including species of *Aspergillus* etc. have been reported as common contaminants of kolanuts. These fungi can produce mycotoxins, such as aflatoxins, which are harmful to human health and can lead to severe health issues, including liver cancer. Fungi are known to colonize kolanuts during growth, harvest, storage, and transportation, leading to spoilage, mycotoxin contamination, and potentially adverse health effects when consumed.

The diseases of stored nuts are the dry rot, grey mould and black rot, caused by *Fusarium solani*, *Botrytis sp* and *Botryodiplodia theobromae.* Grey mould is a serious disease of stored kolanuts, which spread rapidly from nut to nut, the kolanuts are covered with lesions having greyish and powdery mycelia. The black rot is characterized by brownish black encrustations which appear in form of spots over black and hard. Many fungi are capable of infecting kola fruits at an early stage of development, but the disease symptoms will only develop when conditions are favourable. There is need for identifying the fungi responsible for the rots and damages of the fruits to enhance productivity and storage. Understanding the diversity of fungi associated with kolanuts and their potential mycotoxin-producing capabilities is crucial for ensuring the safety and quality of this culturally significant commodity. Moreover, it is essential to develop effective methods for the isolation and identification of these fungi to implement appropriate control measures during cultivation, storage, and processing. By investigating the fungal flora associated with kolanuts and their mycotoxin-producing potential, this research work aims to contribute to the improvement of kolanut safety and quality, ensuring its continued cultural and economic significance in West Africa.

Materials and Methods

Study Area

The study was carried out in Anambra State, Nigeria. It lies within the tropical rain and evergreen forest with a tropical climate that is humid all year round; although the humidity varies with the seasons. The rainy season spans from March to October and is bimodal with a two-week break of rainfall in August (August break). The mean annual rainfall in the Southeast is 2000m while the average annual temperature is between 25^{0}C and 28^{0}C with relative humidity of about 98% during the rainy season and between 50% and 60% during dry season (ADP, 2010).

Source of Materials

Diseased kolanut (*Cola nitida*) samples were collected from four different Markets; Eke-Awka, Nkwo-Amaenyi, Garki-Amawbia, and Kwatta-Amansea all in Awka, Anambra State, Nigeria. They were placed in a sterile polythene bag and brought to the Department of Botany laboratory, Nnamdi Azikiwe University Awka for culturing, isolation, identification and cross innoculation.

Media Preparation

The medium used for the fungal isolation is Potatoes Dextrose Agar (PDA). Ten grams of the powder was dispensed into 100 ml of distilled water in a conical flask and then plugged tightly with cotton wool and foil and heated in water bath for about 2 hours until the agar is melted. The prepared medium was then sterilized using autoclave at 120^{o}C and 30 psi [8, 12] for 15 minutes. Thereafter, it was allowed to cool and then dispensed into the Petri dishes.

Preparation of Sample Inocula

Inocula were prepared from four (4) unhealthy kolanuts. The unhealthy kolanuts were first washed in sterile water and then surface sterilized using 70% ethanol. A sterile kitchen knife was used to cut each of the samples so as to reveal the boundary zone between the rotten and healthy part of the kolanuts. Small bits were cut from the boundary zone of each tuber and transferred to sterile Petri dishes and later used for isolation of fungi pathogens.

Isolation of Test Fungi from Rotten Kolanuts

Isolation of fungi was done by agar dilution plate method. The method was used by [11]. The inoculum prepared from the diseased kolanut was used for isolation of the fungi. Three pieces each of the four different samples of the kolanuts was placed in each Petri dish containing PDA media. All plates were wrapped externally with masking tape and incubated at ±27^{o}C for 72 hours and observed daily for growth of fungi.

Culturing and Identification of Test Fungal Pathogens

Subcultures were prepared using inocula from different organisms in the mixed cultures to obtain a pure culture; this was done by transferring from the colony edge of the mixed cultures to fresh sterile PDA plates with the aid of a scalpel. The plates were wrapped externally with masking tape and incubated for 72 hours. The resulted pure cultures were used for subsequent identification of fungi isolates. The identification was on the basis of their micro and macro- morphological characteristics using standard taxonomic key used previously by [29].

Pathogenicity Test for Kolanuts

Fresh kolanuts were bought and the fungi colonies were inoculated in the fresh healthy samples. Healthy kolanuts weighing 3 grams each were weighed into four places and placed in sterile Petri-dishes. A sterile knife was used to create wounds in the sliced tuber samples and the isolated fungi were incubated into the wounds separately for 7 days and labeled. At the end of the 7th day, the extent of the rot caused by the fungi was determined using the method as described by [14].

Rot (%) = A-a/A x 100

Where:

A = Initial weight of tubers

a = Final weight of tubers after the removal of the rotten portion

Data Analysis

The data obtained was analysed using the statistical package SPSS Version 2023. Data obtained from the study were subjected to Analysis of Variance (ANOVA) at 5% significant level. Means were also separated using Duncan Multiple Range Test.

Results

Occurrence of Fungi Pathogens on Kolanut Samples from Different Locations in Awka

Based on the growth of the fungi on the cultured kolanut specimens, table 1 revealed the presence of *Aternaria alternata, Fusarium oxysporum*, *Aspergillus niger* and *Aspergillus flavus*. *Aspergillus niger* was absent only in kolanut sample from Amansea, thus, having a percentage occurrence of 75%. *Aspergillus flavus* was

Table 1: Occurrence of fungi pathogens on kolanut samples from different locations in Awka.

Fungi Isolates	Eke Awka	Nkwo Amaenyi	First Market	Amansea	Percentage
Aspergillus niger	+	+	+	-	75%
Aspergillus flavus	+	-	+	+	75%
Alternaria alternata	+	+	+	+	100%
Fusarium oxysporum	-	+	-	+	50%

Table 2: Identification of fungi isolates on kolanut samples.

Organism	Macroscopy	Microscopy
Aspergillus niger	Black, powdery colonies with diffused hyphae in media	Smooth-walled stipe; conidiophores radiate and terminate in vesicle
Aspergillus flavus	Light green, powdery colonies	Rough, coarse aerial hyphae; simple sporangiophores shaped globose
Fusarium oxysporum	Wooly, cottony whitish colonies spreading widely	Hyaline (translucent), branched, septate hyphae; 2–6 µm diameter, smooth walls
Alternaria alternata	Whitish colony turning brownish	Brown-black, globose sporangia; rhizoids and zygospores present

Table 3: Pathogenicity of Fungi Isolates on Kolanut Samples.

Fungi Isolates	DAY 4	DAY 5	DAY 6	DAY 7
Aspergillus niger	1.33 ± 0.08^{b}	1.41 ± 0.10^{b}	2.01 ± 0.02^{a}	2.23 ± 0.04^{a}
Aspergillus flavus	2.38 ± 0.04^{c}	3.00 ± 0.08^{bc}	3.22 ± 0.06^{b}	4.20 ± 0.10^{a}
Alternaria alternata	2.53 ± 0.04^{b}	2.60 ± 0.04^{b}	2.64 ± 0.01^{b}	2.70 ± 0.00^{a}
Fusarium oxysporum	2.74 ± 0.06^{b}	2.76 ± 0.05^{b}	2.80 ± 0.08^{a}	2.89 ± 0.04^{a}
LSD	0.242	0.721	0.487	0.559

Results show values of mean of triplicate analysis ± STD. Figures with different alphabets on the same column are significantly different (P<0.05).

present in kolanut samples from all the markets except Nkwo Amaenyi and it had a percentage occurrence of 75%. *Alternaria sp.* was seen in kolanut samples from all locations, giving it a 100% percentage occurrence while *Fusarium oxysporum* was seen in samples of kolanut species collected from Nkwo Amaenyi and Amansea respectively and had a percentage occurrence of 50% (Table 1).

Identification of Fungi Isolates on Kolanut Samples

Based on physical observation of the growth of the fungi on the kolanut specimen and the structures that were observed under the microscope to reveal the type of hyphal growth, fruiting bodies and if present, the types of resting spores, table 2 shows the several fungal species isolated.

Pathogenicity of Fungi Pathogens on Kolanut Samples from Different Locations in Awka

Table 3 shows the result of the pathogenicity test of fungi pathogens isolated from the diseased kolanut samples on the fresh samples. The result showed that all the fungi pathogens had a progressive but slow increase in pathogenicity from day 3 to the 6th day, although *A. flavus* had a higher pathogenicity on the fresh kolanut samples when compared with *Aspergillus niger*. *Alternaria alternata* and *Fusarium oxysporum* showed no significant difference in their pathogenicity on the fresh kolanut sample. *A. niger* had the least pathogenicity in all the days. The pathogenicity of the fungi isolates and the number of days were significantly different (P<0.05).

Discussion

The fungi associated with spoilage in kolanut were identified to be *Aspergillus niger, Aspergillus flavus*, *Alternaria alternata* and *Fusarium oxysporum* (Table 1). The results indicated that the aforementioned fungi reduced the nutritional and mineral compositions of the vegetable [27].

These pathogens showed varying percentage occurrence, ranging from 50%, 75% to 100% indicated that these kolanuts host active fungal contaminants during storage and transport [3].

Both *Aspergillus* and *Fusarium* genera contain many opportunistic pathogens and toxin producers that can diminish crop quality and safety [25]. However, [22] noted that root and tuber crops at time of harvest may already be infested by pathogens derived from disease foliage, roots or mother tubers/cormels. The most prevalent fungus of this research work was *Alternaria alternata,* which is associated with production of ochratoxin A, an immunosuppressive and carcinogenic mycotoxin that persists even in finished food products [15]. Its high frequency in the kolanuts may indicate opportunities for ochratoxin contamination. Similarly, the 75% contamination rates for *A. flavus* and *A. niger* poses concerns due to the ability of these *Aspergillus* species to synthesize the carcinogenic compounds aflatoxins B and G.

Aspergillus, Alternaria, Fusarium and *Mucor* species have been known to bring about rot and spoilage in fruits and vegetables and this has been reported by several authors [17]. Also in line with this, Omayama *et al.* (2010) reported that soft rot of guava was caused by *Alternaria solani* and *R. stolonifer* and thereby resulted in problems during storage and distribution processes; this study conforms with the present study carried out on kolanut bought from different markets in Awka. This study showed that *Alternaria* sp was more prevalent in the samples obtained from all the locations.

More so, the high occurrence of *Alternaria sp* might be due to the report of its ability to grow faster and high pH tolerance, hence, this makes it an important cosmopolitan fungi associated with post-harvest decay and soft rot of different substrate [29]. These organisms are soil saprobes with a wide array of hydrolytic and oxidative enzymes involved in the breakdown of plant lignocelluloses and this is because of their ability to produce extracellular organic acids.

The fungi isolates on kolanuts reported in this study was in concordance with the findings of [10] and [4]. They also reported the occurrence of *Aspergillus niger* and *A. flavus* in their findings. The incidence of these fungi is not restricted to kolanuts only as [2] reported their occurrence in retail cashew nuts also. Some species of the isolated fungi from the kolanuts especially species of *Aspergillus* are known to have strains that produce toxic metabolites; thus, they pose a potential hazard to consumers' health. This observation corroborates the findings of Adebajo and Popoola (2003) which reported the isolation of *Aspergillus niger*, *A. flavus*, and *F. oxysporum* as most commonly isolated fungi from kolanuts. The findings of [10] showed the isolation of fungal species belonging to the genera *Aspergillus* and the subsequent detection of Ochratoxin A (OTA) from the kolanut samples.

The pathogenicity test of the fungi pathogens isolated from the diseased kolanut samples, on the healthy kolanuts showed that there was a progressive increase on all the fungi tested. *Aspergillus flavus* had the highest pathogenicity in amongst all the isolated fungi while *Fusarium oxysporum* had an almost equal pathogencity in all the days. The *Aspergillus niger* showed a slow but steady increase in their pathogenicity, although, it had the lowest pathogenicity generally. These findings differed from the observations by [21] who reported that all the four test fungi they isolated were pathogenic with the most virulent being *Botryodiplodia theobrome*.

Relatively, [29] reported that *Mucor* sp. were pathogenic on some local fruits and vegetables among which were *Psidium guajava, Citrus limon, Mangifera indica, Musa paradisiacal, Phyllanthus emblica, Talinum triangulare* and *Carambola* sp. [18] reported the prevalence of *Aspergillus* sp., also, *Rhizopus* sp on the post-harvest rot of vegetables and fruits. Chuku *et al*. (2002) also observed that A. *niger, P. italicum, R. stolonifer, A. tamarii* and *A. flavus* reduced the viscosity and storage stability of the seed of *Irvingia gabonensis*.

Conclusion

Kolanut is one of the most popular fruits mostly used for various purposes especially for ceremonies. This study has identified the fungi associated with spoilage in kolanut. The work however, showed the fungi that cause this disease and these include *A. niger, A. flavus*, *Fusarium oxysporum* and *Alternaria alternata*. These fungi had fast growth and were spread within a short period of time.

References

1. Adebajo L. O and Popoola O. J. Mycoflora and mycotoxins in kolanuts during storage. African Journal of Biotechnology, 2003, 2(10): 365–15.
2. Adebajo L and Diyaolu S. Mycology and spoilage of retail cashew nuts. African Journal of Biotechnology, 2003, 2(10): 369–373.
3. Adebayo G.J, Kingsley E.O, Daniel A.S, Opeyemi I.J and Toluwalope A.E Mycological Evaluation of Contaminated Kolanuts in Nigeria. Journal of Agricultural Safety and Health, 2021, 27(2): 71–79.
4. Agbeniyi S.O. Post harvest incidence and control of fungi associated with Kolanuts (Cola nitida (Vent) Schott and Endl. And Cola acuminata (Brenan) Schott and Endl.). Department of Biological Sciences, University of Agriculture, Abeokuta, November, 2004. PhD thesis.
5. Asogwa E. U, Anikwe J and Mokwunye F. C. Kola production and utilization for economic development. African Scientist, 2006, 7(4): 217-222.
6. Atanda S.A, Pessu P.O, Agoda S, Isong I.U and Ikotun I. The Concepts and Problems of Post-harvest Food Losses in Perishable Crops. African Journal of Food Science, 2011, 5: 603-6013.
7. Atawodi S. E. O, Fundstein B. P, Haubner R, Spiegelhalder B, Bartsch H, Owen R. W. Content of polyphenolic compounds in the Nigerian stimulants Cola nitida sp alba, Cola nitida sp. rubra A. Chev, and Cola acuminata Schott and Endell and their antioxidant capacity. Journal of Agriculture and Food Chemistry, 2007, 55: 9824-9828.
8. Cheesbrough, M. Microbiological Tests. In: Cheesbrough, M., Ed., District Laboratory Practice in Tropical Countries, Part II, Low Priced Edition, Cambridge University Press, Cambridge, 2000, 105-130.
9. Chuku E.C, Onuegbu B.A and Osakwe F.C. Mycoflora of Irvingia gabonensis var. gabonensis (Ugiri), their incidence and seed rots. African Journal of Science and Technology, 2002, 3: 54-55.
10. Dongo L. N, Manjula K and Orisajo B. Occurrence of ochratoxin A in Nigerian kolanuts. African Crop Science Conference Proceedings, 2007, 8: 2133-2135.
11. Humaidi F, Abadi A.L and Siti R. Thiophanate-Methyl Fungicide Residues Levels in Soil on Potato and Life Impact on Soil Fungi in the Stone Malang. 1999. http://www.peipfikomdasulsel.org/wp content/uploads/2012/04. Retrieved April 2024.
12. Jawetz M.A, Brooks G.F, Butel J.S and Morse S.A. Medical Microbiology. 23rd Edition, McGraw Hill Companies Inc., Singapore, 2004, 818 p.
13. Jayeola C. O. Preliminary Studies on the use of kolanuts (Cola nitida) for soft drink production. Journal of Food Technology in Africa, 2001, 6(1): 25-26.
14. Kassim M. Y. Chemical control of post-harvest disease of potato and tomato. Indian Phytopathology, 1986, 2: 343-344.
15. Krishnamurthy Y.L, Shashikala J and Sharma S. Fungal and aflatoxin contamination of marketed cashew nuts, pistachios and walnuts. Food Additives and Contaminants - Part B Surveillance, 2009, 2(3): 194-198.
16. Lovejoy P. E. Kola in the History of West Africa. Cahier d'Etudes Africaines. 1980, 77-78: 97-134.
17. Makun H. A, Gbodi T. A, Akanya H. O, Sakalo A. E and Ogbadu G. H. Health implications of toxigenic fungi found in two Nigerian staples: guinea corn and rice. African Journal of Food Science. 2009, 3: 250-256.
18. Makun H. A, M. F, Dutton P. B, Njobeh M, Mwanza and Kabiru A. Y. Natural multi- mycotoxin occurrence in rice from Niger State, Nigeria. Mycotoxin Research. 2011, 27(2): 97-104
19. Mokwunye F. C. Functional characterisation of kola nut powder for beverage production. M.Sc. dissertation. University of Agriculture, Abeokuta. Nigeria. 2009, Pp. 1-69.
20. Ndagi I, Babalola F.D, Mokwunye I.U, Anagbogu C.F, Aderolu I.A, Ugioro O, Asogwa E.U, Idrisu M and Mokwunye F.C. Potentials and Challenges of Kolanut Production in Niger State, Nigeria. International Scholarly Research, 2012: 1-9.
21. Okigbo R. N and Nnadiri P. C. Effect of Three Tropical African Plants on some Fungal Rot of Stored Cocoyam (Colocasia esculenta L). International Journal of Agricultural Technology, 2017, 13(2): 183-203.
22. Okigbo R. N, Nmeka I. A. Control of yam tuber rot with leaf extracts of Xylopia aethiopia and Zingiber officinale. African Journal of Biotechnology, 2005, 4(8): 804-807.
23. Omayma M. I, El-Moniem E. A. A. A, Abd-Allah A. S. E and El-Naggar M. A. A. Influence of some post-harvest treatments on guava fruits. Agriculture and Biology Journal of North America, 2010, 1(6): 1309-1318.
24. Opeke L. K. Tropical Commodity Tree Crops. Spectrum Books Sushine House: Ibadan. 2005, 80p
25. Perrone G, Susca A, Cozzi G, Ehrlick K, Varga J, Frisvad J.C, Meijer M, Noonim P, Mahakarnchanakul W and Samsom R.A. Biodiversity of Aspergillus species in some important agricultural products. Study in Mycology, 2007, 59: 53-66.

26. Petu-Ibikunle A. M, Esther W, Audu A and Shallagwa Y. Y. Assessment of damage and losses to kola nut weevils, Balanogastris kolae (Desbr) Coleoptera: Curculionidae. African Journal of General Agriculture, 2010, 6(1): 1-5.

27. Revati R, Nalawade R. R, Rathod R. R, Kalaskar M. S, Joshi J. Joy, Nirgude Y.K, Phondekar U.R and Amruta D. G. Investigation of Fungi Associated with Dried Copra and Coconut Oil. International Journal of Current Microbiology and Applied Science, 2019, 8(10): 1729-1734.

28. Russell T. A. The kola of Nigeria and the Cameroons. Tropical Agriculture, Trinidad, 1955, 32: 210-240.

29. Samson R. Am, Varga J and Dyer P. S. Morphology and Reproductive Mode of Aspergillus fumigatus, In Latga, J. P. & Steinbach, W. J. (Eds.) Aspergillus fumigatus and aspergillosis, (Washington, DC, ASM Press, 2009). 2010.