MYCOFLORA, AFLATOXIN ASSESSMENT AND SHELF-LIFE STUDY OF *Cyperus esculentus* (TIGERNUT) AND TIGERNUT MILK USING *Cymbopogon citratus*

BY

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19PCQ02045

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc) DEGREE IN MICROBIOLOGY IN THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF SCIENCE AND TECHNOLOGY, COVENANT UNIVERSITY

OCTOBER, 2021

ACCEPTANCE

This is to attest that this dissertation is accepted in partial fulfilment of the requirements for the award of Master of Science Degree (MSc.) in Microbiology in the Department of Biological Science, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria.

Mr. John A. Philip

(Secretary, School of Postgraduate Studies) Signature and Date

Prof. Akan B. Williams

(Dean, School of Postgraduate Studies) Signature and Date

DECLARATION

I, **ALADE, MAZEEDAT BOLUWATIFE 19PCQ02045**, declare that this research titled **'MYCOFLORA, AFLATOXIN ASSESSMENT AND SHELF-LIFE STUDY OF *Cyperus esculentus* (TIGERNUT) AND TIGERNUT MILK USING *Cymbopogon citratus*"** was carried out by me under the supervison of Prof. Obinna C. Nwinyi of the Department of Biological Sciences, College of Science and Technology, Covenant University. I attest that the dissertation has not been presented either wholly or partially for the award of any degree elsewhere. All the sources of data and scholarly publications used in this dissertation are duly acknowledged.

ALADE, MAZEEDAT BOLUWATIFE ……..

Signature and Date

CERTIFICATION

We certify that this dissertation titled **'MYCOFLORA, AFLATOXIN ASSESSMENT AND SHELF-LIFE STUDY OF *Cyperus esculentus* (TIGERNUT) AND TIGERNUT MILK USING *Cymbopogon citratus*'** is an original research work carried out by **ALADE, MAZEEDAT BOLUWATIFE (19PCQ02045)** in the Department of Biological Sciences, College of Science and Technology, Covenant University under the supervsion of Prof. Obinna C. Nwinyi. We have examined and found this work acceptable as part of the requirements for the award of Master of Science in Microbiology.

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(Supervisor) **Signature and Date**

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(Head of Department) **Signature and Date**

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(External Supervisor) **Signature and Date**

Prof. Akan B. William .....

(Dean, School of Postgraduate Studies) **Signature and Date**

DEDICATION

To almightly God I gave Him all the glory for His help and strength. For the right people you kept at every step of the way, thank you Lord. There was no way I would have made it without your word which served as a lamp and light to my feet and path.

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LIST OF ABBREVIATIONS

DEP-	Dichloromethane: ethyl acetate: propan-2-ol
HPLC-	High performance liquid chromatography
ITS-	Internal Transcribed Spacer
MEGA 7-	Molecular Evolution Genetic Analysis version 7.0
NCBI -	National Center for Biotechnology Information
NJ-	Neighbour Joining method
PBS-	Phosphate buffered saline
PCR-	Polymerase chain reaction
RF-	Retention factor
SPE-	Solid phase extraction
TEF-	Toluene/ ethyl acetate/formic acid
TLC-	Thin layer chromatography

ABSTRACT

Aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus*, are secondary metabolites that pose a major threat to global food security. This could result in detrimental impacts on human and animal health. In this study, the presence of aflatoxigenic fungi and aflatoxins in *Cyperus esculentus* L. (tigernut) seeds and tigernut milk were assessed. Also, the shelf extension using *Cymbopogon citratus* (lemongrass) was determined. Samples were obtained from three major dealers in Ota, Ogun State using the snowball sampling technique. The seeds were cleaned and processed into tigernut milk, thereafter stored for further studies. The organisms were identified using morphological and molecular methods using the internal transcribed spaces ITS 1 and ITS 4. Qualitative and quantitative assessments of the toxigenic potential of the fungi were carried out using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) methods respectively. For the shelf-life study of tigernut milk, three different formulations were used (10ml, 15ml and 20 ml of lemon grass extract). Data obtained from this study were analyzed using descriptive statistics in form of mean and standard deviation. Total fungal count in tigernut and tigernut milk ranged from 1.47×10^3cfu/g - 6.94×10^5 cfu/ml. The predominant fungi in both samples were *Aspergillus flavus* (OK172340), *Aspergillus orzyae* (OK172339) and *Aspergillus brasiliensis* (OK17234). The aflatoxin levels detected ranged between 1.4 - 2.03ppb in tigernut samples while the tigernut milk samples were free of aflatoxin. Total fungal counts of the fortified tigernut milk ranged between 9.9×10^5 -1.28×10^6 cfu/ml. The fungal isolates identified over the 14 days shelf-life storage were *Aspergillus* spp., *Penicillium* spp. and *Rhizopus* spp. for each day. From the results obtained from the test panel, there was a general acceptance of the sensory quality; LA104 had higher general acceptance while LA267 had the least acceptance. The shelf-life was extended for 14 days without loss in the organoleptic properties of the tigernut milk. In conclusion, the tigernut and tigernut milk analyzed had aflatoxin content below the acceptable limit 4ppb with respect to National Agency Food and Drug Administration and Control (NAFDAC) guidelines. In addition, lemongrass improved the shelf life of tigernut milk over a period of 14 days under refrigerated condition.

Keywords: Aflatoxins, *Cympbogon citratus*, HPLC, Tigernut, TLC