# EFFICACY OF SELECTED SPICES IN MITIGATION OF FUNGAL GROWTH AND MYCOTOXINS PRODUCTION IN TEA AND COFFEE

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# EFFICACY OF SELECTED SPICES IN MITIGATION OF FUNGAL GROWTH AND MYCOTOXINS PRODUCTION IN TEA AND COFFEE

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## CERTIFICATION

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## DEDICATION

This effort is dedicated to God, the giver of life, reservoir of knowledge and crowner of success.

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## ABSTRACT

Tea and coffee, consumed for their neutraceutical, health and stimulating benefits, can be easily contaminated by mycotoxin-producing fungi, due to agro-ecological factors, poor handling and storage. Spices such as *Curcuma longa*, *Xylopia aethiopica* and *Piper guineense* possess antimicrobial properties with potential to reduce fungal growth in tea and coffee. However, there is limited information on their use and effectiveness in mitigating mycotoxins production in processed tea and coffee. This study was designed to investigate the extent of fungal contamination and possibility of inhibiting growth of fungi and their metabolites in tea and coffee using the selected spices.

Tea and coffee, randomly obtained from fourteen warehouses each in Ibadan and Mambilla, were subjected to microbial screening and mycotoxins quantitation following ISO methods. Fungal isolates were characterised using RAPD analysis, and metabolites recovered were quantified using HPLC. Freshly harvested tea and coffee from Ibadan and Mambilla were processed to green and black products using standard procedures, and treated with milled *Curcuma longa* roots, as well as *Xylopia aethiopica* and *Piper guineense* seeds at 1000, 2000, 3000 and 4000 µg/g. The treated and untreated (control) samples were packaged in tea bags and stored at ambient condition (28±3$^{o}$C; 70±5% Relative Humidity) for 24 weeks. During the storage period, microbial activities were monitored and polyphenol levels determined bi-weekly using AOAC methods. Data were analysed using ANOVA at $\alpha_{0.05}$.

*Aspergillus flavus*, *Aspergillus niger*, *Penicillium georgiense*, *Fusarium solani* and *Gliocladium cibotti* were prominent fungal species identified in the tea and coffee. The tea and coffee contained metabolites of Aspergillus (15, 2), Penicillium (8, 6), Fusarium (4, 5) and other fungal species (18, 12). Mambilla tea and coffee had significant higher level of contamination than Ibadan samples. Seventy toxins were quantified, with Aflatoxin $B_1$ (85.3-427.2 µg/kg), deoxynivalenol (1.3-5.2 µg/kg), beauvericin (2.5-5.0 µg/kg), brevianimide (33.6-106.2 µg/kg), and sporidesmolide II (7.3-10.8 µg/kg), being major in the tea and coffee. Aflatoxin $B_1$ and deoxynivalenol, which are of major public health concern, were dominant in black tea and coffee. Green tea and coffee were also contaminated with abscisic acid, unspecific rugulusovin, sporidesmolide II, unspecific emodin and unspecific tryptophol. *Curcuma longa* (4000 µg/g), *Piper guineense* (4000 µg/g) and *Xylopia aethiopica* (1000 µg/g) had 92.0±1.1%, 92.0±1.8% and 91.3±2.4% microbial activity reduction on *Aspergillus flavus* and 53.2±0.8%, 36.4±1.5% and 37.8±2.7% on *Fusarium solani*, respectively. Microbial inhibition of the spices varied significantly with concentration. The source of samples did not significantly influence the efficacy of the treatment. Total phenolic content increased with spice addition, in black tea and coffee (3.0-7.1 mg/g), and in green tea and coffee (15.5-22.0 mg/g), respectively.

High number of Fungi species and their metabolites were present in the tea and coffee. *Xylopia aethiopica* was most effective in reducing fungal growth and mycotoxin production in the tea and coffee.



**Keywords**: Tea and coffee, Spices, Fungal metabolites, Mycotoxin

**Word count**: 465

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| | |
|---|---|
| **AOAC** | Association of Analytical Chemists |
| **CLS** | Curcuma longa spice |
| **XAS** | Xylopia aethiopica spice |
| **PGS** | Piper guineense spice |
| **$a_w$** | Available water |
| **$AfB_1$** | Aflatoxin $B_1$ |
| **DON** | Deoxynivalenol |
| **PCR** | Polymerase Chain Reaction |
| **LoD** | Limit of Detection |
| **ND** | Not Detected |
| **HPLC** | High Performance Liquid Chromatography |
| **EEC** | European Economic Commission |
| **FT-IR** | Fourier Transform Infra Red |
| **BBD** | Box Behnken Designs |
| **GFF** | General Full Factorial |
| **TPC** | Total Phenolic Content |
| **TDI** | Tolerable daily intake |
| **t-TDI** | Total Tolerable daily intake |
| **µg/kg** | microgramme per kilogram |
| **ng/g** | nanogramme per gram |
| **µg/g** | microgramme per gram |
| **pg/g** | picogramme per gram |
| **PDA** | Potato-dextrose agar |
| **YES** | Yeast extract sucrose |
| **RNA** | Ribonucleic acid |
| **DNA** | Deoxy ribonucleic acid |
| **MAS** | Marker assisted selection |
| **QTL** | Quantitative trait loci |
| **sMRM** | Scheduled multiple reaction monitoring |

| | |
|---|---|
| **MS** | Mass spectrometry |
| **Bw/day** | body weight per day |

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of Study

*Camellia sinensis* (L) Kuntze, tea, was imported into Nigeria in 1972 from Kenya by Nigerian Beverage Production Company (NBPC). Information within reach revealed that 2045, 2914, and 1810 tonnes of tea were made in Nigeria in 2006, 2007 and 2018 respectively (Olaniyi *et al*., 2014; Ipinmoroti *et al*., 2018). Coffee (arabica, robusta, liberica), on the other hand, ranks after tea in terms of popularity as a beverage (Aderolu *et al.*, 2014). These beverage crops, tea and coffee, apart from being a mainstay in the daily diet of Nigerians, Africans and by extension globally; are important export commodity for countries producing them. While tea (*Camellia sinensis*) is native to India, China, Japan, Srilanka, Vietnam, Indonesia (countries in Asia), and African countries such as Kenya, Nigeria, Uganda and Malawi, Africa uniquely gave coffee to the world (Olaniyi *et al.*, 2014).

Nearly sixty to eighty percent (60-80%) of all agricultural produce especially staple food crops are rendered unfit for use by mycotoxins like aflatoxins, fumonisins, ochratoxin A, deoxynivalenol and zearalenone (Adebo *et al.*, 2021). In Africa, it is evident, the negative impact of crop contamination on trade with European Economic Commission (EEC). Atanda and his colleagues (2011), reported a decline by fifty percent (50%) on import of peanut meal by the EEC in 1980 – 1990 due to strong regulation of aflatoxin. Contamination of agricultural commodities by fungi metabolites and the subsequent impact on consumer health and consequently on proceed from local and global trade is increasingly recognized by developed and developing nations. This therefore makes management of naturally occurring toxins, like mycotoxins, a unique challenge in ensuring safe and wholesome food is available world-wide (Atanda, 2011). Developed countries have imposed regulatory laws, setting threshold for mycotoxins

contamination in foods (Ahmed *et al.*, 2022), shielding unsuspecting partners from being exposed to risks of mycotoxins whereas in many developing nations, several agricultural commodities are still unregulated and where regulatory guidelines exists, economic incentives for high quality products free from mycotoxin contamination are not implemented (Makhuvele *et al.*, 2020).

Many agricultural commodities used in food and beverage production may be contaminated by mycotoxinogenic fungi, which synthesize mycotoxins, as a result of poor agricultural practices, inadequate knowledge of processing, poor handling, packaging, transport and storage conditions. Uncontrolled fungal growth, aided by poor storage conditions, may increase risk of mycotoxin contamination (Marin *et al*., 2013). Often, soiled commodities include cereals, tea, coffee, peanuts, beer, wine, vegetables, nuts, cottonseeds, fresh and dried fruits and dairy products. For example, researchers have assiduously studied traditionally prepared African beverages made from local raw materials and in most cases, reported the presence of fungal toxins (Lei *et al*., 2016; Ezekiel *et al*., 2018). Nowadays, since these products are widely used both as food and medicine, they enjoy wide range of presentation, from green to white to fruit-based infused teas. Today herbal teas are very popular (Zhao *et al*., 2016). A team comprising Chinese research fellows, analyzed tea and reported insignificant risk regarding $FB_1$ and T-2 toxin, although, a sizeable number of samples did exceed agreed thresholds for $AFB_1$ and DON (Adebo *et al*., 2021; Wu *et al*., 2014).

## 1.2 Statement of the problem

During handling and storage of processed tea and coffee, contamination as a result of toxigenic mold may occur (Emmanuel *et al.*, 2020). Moreover, environment, often time is a potential carrier of bacteria and mould spores (Lopez *et al.*, 2018). Need therefore arises for prevention of mycotoxigenic mould and their metabolites from growing on processed tea and coffee and when it grows, find a means to suppress production of harmful metabolites from reaching potential consumers.

## 1.3 Objectives of the Research

The objectives of this work were:

i. To study the effect of some tropical spices on chemical composition of tea and coffee

ii. To detect mycotoxigenic fungi present in tea and coffee vis-a-vis quantify mycotoxin contaminants found in stored tea and coffee

iii. To investigate the effectiveness of *Curcuma longa, Xylopia aethiopica and piper guineense* powder against aflatoxin ($AfB_1$) and deoxynivalenol (DON) contaminants in stored tea and coffee

iv. To evaluate the sensory attributes of produced spiced-beverage.

## 1.4 Justification for the Research

Conventional approach namely, physical, chemical and biological strategies to reduce fungi growth and detoxifying mycotoxins to a safe level, are not without limitations such as, significant alterations in the food colour, flavor, texture and nutritional contents (Haque *et al*., 2020). Moreover, the concern of thermal stability of mycotoxins and chemical residue while deploying conventional strategies for food processing (Mokhtarian *et al*., 2020; Okeke *et al*., 2018), necessitate increased interest in innovative approach devoid of direct application of heat on food matrices, which will midwife notable reduction in amount of mycotoxin in foods without impairing its organoleptic quality. The deployment of botanicals, rich in naturally occurring phenolic compounds becomes promising in reducing mycotoxins contamination in food (Ahmed *et al*., 2022). Spices abound in the tropics. They are cheap and densely laden with phenolic compounds (Aroyeun *et al*., 2011). Uses of tropical spices have been considered as safe for humans due to their anti-fungal and anti-mycotoxin properties thus increased level of acceptability. The effect of inclusion of these spice inhibitors on tea and coffee quality requires investigation and development of cheap and affordable technological interventions; in response to increasing preference for safe foods, will help improve the safety of beverages, made from infected tea and coffee.

# CHAPTER TWO

# LITERATURE REVIEW

This literature focuses on tea and coffee, which have high acceptance across the globe, their uses, the different mycotoxin contaminants especially aflatoxins (Aflatoxin $B_1$) and trichothecenes (deoxynivalenol) and documented ways of reducing this fungi metabolites.

## 2.1 Tea

Tea, leave of *Camellia sinensis* plant, is a popular and widely relished beverage for its characteristic antioxidant property and specific aroma, considered suitable for human wellness around the world (Li *et al.*, 2013). Tea is an everyday drink cherished by everyone, an evergreen bush, largely grown in East Asia and prepared by hot water infusion of the cured leaves of the camellia. Its consumption has a growing outlook among the middle class of the urbanized population and within the new order societies of developing markets (Chang, 2015; Hilal and Mubarak, 2013). Tea also referred to as "the queen of beverages" due to its ranking as the second most-ingested liquid aside water, by producing countries alongside her importers, who sees it as a valuable plant and a source of income. Countries like Turkey, Sri Lanka, Kenya, India and China cash in on tea production (Sereshti *et al*., 2013). The tea genus, Camellia, of the *Theaceae* family, has only two primary tea varieties: *Camellia sinensi* var. *sinensis* and *Camellia sinensis* var. *assamica* and aside from consumption, tea is also grown on a global scale for its economic value. According to FAO (2015), about 4.73 million tones of tea were produced in 2008. The health benefits of tea, including cancer prevention, attracted it to researchers and have been studied extensively. Nevertheless, different confounding factors have hindered swift conclusions on epidemiological studies on the anticancer effect of consuming tea by humans and today, teas have been commercially modified to include green tea, white tea, and fruit-enhanced infused tea. Infused drinks prepared from herbal

sources are much popular because of their nutritional and medicinal value (Zhao *et al.*, 2016).

### 2.1.1 World tea production

In 2700 B.C, the Chinese discovered the tea plant (*Camellia sinensis* (L.) *Kuntze*) of the *Theaceae* family and it is regarded as the most widely drunk non-alcoholic drink on a global scale (Chang, 2015) and grown in about 5 of the 7 continents in the world. The genus, Camellia L., reportedly has over 200 species, but the tea stands out as the most commercially viable plant. It is a woody plant with evergreen foliage. During cultivation, the tea tree is purposively grown at a low level for easy young shoot harvesting for tea beverage drink production (Chang *et al*., 2021). Compared with other equally viable and thriving global agricultural industries, the tea industry is highly stable.

According to FAO (2018), the global tea production increased from 3.21 million tonnes in 2006 to 4.73 million tonnes in 2008 with countries such as China, Kenya, India, and Sri Lanka leading others. Table 2.1 compares tea production in Nigeria with three other top-producers in the world. Table 2.2 compares the actual volume of tea produced between 2002 up until 2016 and the projected volume of tea in year 2024.

### 2.1.2 History and development of tea in Nigeria

Cultivation of tea has come of age in Africa since early 1900's in Kenya. At present, Kenya occupies the first position among the league of African tea producing countries like Malawi, Burundi, Cameroun, Nigeria etc. Tea production in Nigeria began after the Nigerian Beverage Production Company introduced it into the country from Kenya in 1972 (Olaniyi *et al.,* 2014). In some African countries, such as Kenya, Tanzania, Malawi, and Cameroon, tea plays a vital role in strengthening the national economy. However, the case is slightly different in Nigeria as tea production has not sufficiently equaled its demand by various processing companies. In this wise, local tea production accounts for only about 10% of total in-country consumption while the rest are derived from importation (Ipinmoroti *et al.,* 2018). Nevertheless, tea production has great potentials for poverty alleviation and food insecurity eradication with the right amount of investment in its improvement.

**Table 2.1**: **Tea production comparison among Nigeria and three other top-producers in the world in tonnes**

| **Country** | **2006** | **2007** | **2008** |
|---|---|---|---|
| China | 1,047,345 | 1,183,002 | 1,257,384 |
| India | 9,280,000 | 949,220 | 805,180 |
| Kenya | 310,580 | 369,600 | 345,800 |
| Nigeria | 2,045 | 2,914 | - |

**Source: Olaniyi *et al*. (2014)**

**Table 2.2: Black Tea Production around the World**

| Country/Region | Production | | | | |
|---|---|---|---|---|---|
| | Actual | | Projected | Growth rate | |
| | 2002-2004 | 2012-2014 | 2024 | 2002-04/2012-14 | 2012-14/2024 |
| | (tonnes) | | | (% per year) | |
| **Worldwide** | 2,324,425 | 3,000,764 | 4,294,815 | 2.6 | 3.7 |
| **Africa** | | | | | |
| Kenya | 301,794 | 415,707 | 602,969 | 3.3 | 3.8 |
| Malawi | 43,656 | 44,936 | 47,585 | 0.3 | 0.6 |
| Zimbabwe | 21,080 | 8,500 | 11,442 | -8.7 | 3 |
| Rwanda | 14,870 | 24,884 | 44,692 | 5.3 | 6 |
| South Africa | 7,769 | 2,104 | 5,089 | -12.2 | 9.2 |
| Uganda | 35,775 | 61,424 | 103,645 | 5.6 | 5.4 |
| U.R. Tanzania | 29,227 | 33,824 | 36,037 | 1.5 | 0.6 |
| Others | 34,993 | 39,602 | 53,305 | 1.2 | 3 |
| **Latin America and Caribbean** | | | | | |
| Argentina | 70,015 | 78,522 | 92,228 | 1.2 | 1.6 |
| Brazil | 8,054 | 7,267 | 6,568 | -1.0 | -1.0 |
| Others | 9,567 | 8,718 | 7,004 | -0.9 | -2.2 |
| **Near East** | | | | | |
| Islamic Rep. Iran | 52,617 | 26,000 | 25,978 | -6.8 | 0.0 |
| Turkey | 171,667 | 232,665 | 258,076 | 3.1 | 1.0 |
| **Far East** | | | | | |
| India | 861,205 | 1,164,043 | 1,519,542 | 3.1 | 2.7 |
| Sri Lanka | 306,404 | 331,243 | 402,253 | 0.8 | 2.0 |
| China | 41,999 | 159,211 | 673,613 | 14.3 | 15.5 |
| Viet Nam | 71,047 | 92,832 | 108,002 | 2.7 | 1.5 |
| Bangladesh | 55,973 | 63,787 | 79,253 | 1.3 | 2.2 |
| Malaysia | 3,649 | 19,606 | 33,525 | 18.3 | 5.5 |
| Nepal | 9,122 | 17,300 | 21,255 | 6.6 | 2.1 |
| Indonesia | 125,077 | 112,131 | 115,821 | -1.1 | 0.3 |
| Others | 34,213 | 42,377 | 41,294 | 2.2 | -0.3 |
| Russian Federation | 2,567 | 3,400 | 3,098 | 2.9 | -0.9 |
| Other CIS | 5,192 | 4,200 | 5,167 | -2.1 | 2.1 |
| Oceania | 6,867 | 6,467 | 5,927 | -0.6 | -0.9 |

**Source: FAO-UN, 2018**

### 2.1.3 World tea consumption outlook

The demand for tea responds to many factors. These include income, price, and demographic descriptions such as occupation, age, culture, and educational exposure. Furthermore, the influence of health on tea consumption cannot be over-emphasized. Tea consumption invariably increases as people become more aware of its potential health benefits and the health-hazardous nature of carbonated drinks. Therefore, the economic value of tea has greatly increased, more or less benefitted from the downward slope of regular and diet soda, fruit, and milk beverages sales. This phenomenon has motivated a stronger publicity on the health benefits of drinking tea using an international promotion platform by the IGG/Tea (FAO-UN, 2018).

Considering tea consumption trend from 2006 to 2016, there has been a noticeable annual increase of 4.5% to 5.5 million tons (Table 2.2). The underlying reason for this rapid increment is none other but the increased per capital income in countries like India, China, and many other emerging and growing economies. Many tea-producing countries, such as those in Latin America, Africa, and Asia, likewise experienced significant increase in demand. Notably, consumption levels leaped in China (Table 2.3) annually at 10.1% over ten years to rest at 2.1 million tons in 2016 (accounting for 38.6% of global consumption). India came on China's heels with 1.05 million tonnes consumption level in 2016 (about 19.0% of global consumption (FAO-UN, 2018).

Meanwhile, the Food and Agricultural Organisation (2018) has projected an annual growth rate of 2.5% for black tea consumption until 2027 to reach 4.17 million tonnes. This projection implies a positive growth in tea-producing countries consumption levels, which should compensate for the declining importation rate for consumption-only countries. With an annual increase of 5.9% over the next decade, China is expected to lead others in tea consumption expansion. African countries are also projected to experience higher consumption rates in the following order: Rwanda (9%), Uganda (5%), Kenya (4.4%), Libya (4.4%), Morocco and Malawi (4.2% each). In the same vein, other tea producing nations are expected to grow moderately with about 2 to 3.5%. Such countries include Bangladesh (3.1%), India (2.2%), Sri Lanka (3.3%), Tanzania (1.8%), and Vietnam (2.0%). Lastly, developed western nations are expected to have the lowest

consumption increase rates. Specifically, the UK is expected to have a negative slope trend for black tea consumption amidst greater demand for alternatives, including coffee (FAO-UN, 2018).

**Table 2.3 Green Tea Production and Export**

| Countries/Region | Production | | | | | Exports | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Actual | | Projected | Growth rate | | Actual | | Projected | Growth rate | |
| | 2002-04 | 2012-14 | 2024 | 2002-04/ 2012-14 | 2012-14/ 2024 | 2002-04 | 2012-14 | 2024 | 2002-04/ 2012-14 | 2012-14/ 2024 |
| | (tonnes) | | | (Percent per year) | | (tonnes) | | | (Percent per year) | |
| World | 774,618 | 1,567,092 | 3,743,566 | 7.3 | 9.1 | 211,325 | 344,280 | 804,300 | 5.0 | 8.9 |
| China | 576,580 | 1,315,230 | 3,221,897 | 8.6 | 9.4 | 182,792 | 254,106 | 481,508 | 3.3 | 6.6 |
| Japan | 91,667 | 83,297 | 94,588 | -1.0 | 1.3 | 841 | 2,936 | 8,394 | 13.3 | 11.1 |
| Viet Nam | 34,100 | 95,502 | 339,665 | 10.8 | 13.5 | 16,800 | 64,542 | 284,912 | 14.4 | 16.0 |
| Indonesia | 40,533 | 37,300 | 39,790 | -0.8 | 0.6 | 4,252 | 11,810 | 19,370 | 10.8 | 5.1 |

**Source: FAO-UN, 2018**

**Table 2.4a: Black Tea Consumption**

| Country/Region | Production Actual (tonnes) 2002-04 | 2012-14 | Projected (tonnes) 2024 | Growth rate (tonnes) 2002-04/2012-14 | 2012-14/2024 |
|---|---|---|---|---|---|
| **Global** | 2,275,355 | 2,971,307 | 4,265,506 | 2.7 | 3.7 |
| | | | **Far East** | | |
| Parkistan | 110,107 | 130,575 | 157,472 | 1.7 | 1.9 |
| India | 706,967 | 976,663 | 1,313,191 | 3.3 | 3.0 |
| Sri Lanka | 23,995 | 31,730 | 49,520 | 2.8 | 4.6 |
| China | 4,997 | 140,371 | 584,379 | 39.6 | 15.3 |
| Viet Nam | 45 | 207 | 331 | 16.5 | 4.8 |
| Bangladesh | 40,772 | 62,370 | 93,679 | 4.3 | 4.2 |
| Malaysia | 14,445 | 36,481 | 59,942 | 9.7 | 5.1 |
| Nepal | 8,810 | 8,872 | 9,781 | 0.1 | 1.0 |
| Indonesia | 36,923 | 36,238 | 55,528 | -0.2 | 4.4 |
| | | | **Africa** | | |
| Kenya | 12,968 | 27,158 | 49,126 | 7.7 | 6.1 |
| Malawi | 1,281 | 4,236 | 10,873 | 12.7 | 9.9 |
| Morocco | 163 | 167 | 327 | 0.2 | 7.0 |
| Zimbabwe | 4,762 | 2,600 | 4,758 | -5.9 | 6.2 |
| Rwanda | 1,287 | 1,541 | 1,069 | 1.8 | -3.6 |
| South Africa | 17,052 | 22,140 | 30,453 | 2.6 | 3.2 |
| Uganda | 1,463 | 4,700 | 8,489 | 12.4 | 6.1 |
| U.R. Tanzania | 6,545 | 5,684 | 7,884 | -1.4 | 3.3 |
| Others | 59,029 | 76,764 | 100,489 | 2.7 | 2.7 |

**Source: FAO-UN, 2018**

**Table 2.4b: Black Tea Consumption**

| Country/Region | Production Actual (tonnes) 2002-04 | 2012-14 | Projected (tonnes) 2024 | Growth rate (tonnes) 2002-04/2012-14 | 2012-14/2024 |
|---|---|---|---|---|---|
| | | | **Near East** | | |
| Islamic Rep. of Iran | 76,198 | 83,118 | 119,309 | 0.9 | 3.7 |
| Turkey | 168,248 | 234,037 | 262,856 | 3.4 | 1.2 |
| Iraq | 49,564 | 34,517 | 34,104 | -3.6 | -0.1 |
| Saudi Arabia | 21,162 | 30,843 | 46,767 | 3.8 | 4.3 |
| Syria | 30,041 | 30,333 | 31,547 | 0.1 | 0.4 |
| U.A.E | 39,286 | 40,000 | 66,304 | 0.2 | 5.2 |
| Egypt | 70,016 | 97,402 | 139,675 | 3.4 | 3.7 |
| Libya | 10,294 | 13,227 | 11,628 | 2.5 | -1.3 |
| Sudan | 14,667 | 25,900 | 50,715 | 5.9 | 7 |
| Jordan | 4,843 | 5,404 | 5,945 | 1.1 | 1 |
| Isreal | 1,870 | 1,065 | 1,556 | -5.5 | 3.9 |
| | | | **Latin America and Caribbean** | | |
| Argentina | 7,950 | 5,056 | 5,120 | -4.4 | 1 |
| Brazil | 4,478 | 6,961 | 10,527 | 4.5 | 4.2 |
| Others | 27,327 | 32,482 | 37,841 | 1.7 | 1.5 |
| | | | **North America** | | |
| U.S.A | 83,086 | 107,511 | 144,155 | 2.6 | 3 |
| Canada | 15,113 | 13,030 | 12,598 | -1.5 | -0.3 |

**Source: FAO-UN, 2018**

**Table 2.4c: Black Tea Consumption**

| | Production | | | | |
|---|---|---|---|---|---|
| | Actual | | Projected | Growth rate | |
| Country/Region | 2002-04 | 2012-14 | 2024 | 2002-04/2012-14 | 2012-14/2024 |
| | (tonnes) | | | (percent per year) | |
| | | | Europe | | |
| E.U (28) | 228,083 | 196,591 | 190,302 | -1.5 | -0.3 |
| U.K | 128,987 | 112,174 | 100,375 | -1.4 | -1.1 |
| Germany | 19,310 | 21,942 | 28,901 | 1.3 | 2.8 |
| Poland | 26,797 | 15,343 | 11,821 | -5.4 | -2.6 |
| Netherland | 13,867 | 7,854 | 12,641 | -5.5 | 4.9 |
| France | 8,322 | 7,495 | 7,045 | -1.0 | -0.6 |
| Other E.U | 19,624 | 24,605 | 22,031 | 2.3 | -1.1 |
| Others | 4,151 | 3,037 | 2,292 | -3.1 | -2.8 |
| | | | CIS | | |
| Russian Federation | 154,391 | 151,905 | 139,371 | -0.2 | -0.9 |
| Other CIS | 64,019 | 97,154 | 160,739 | 4.3 | 5.2 |
| Japan | 15,567 | 15,910 | 17,243 | 0.2 | 0.8 |
| Oceania | 16,321 | 15,170 | 13,973 | -0.7 | -0.9 |

**Source: FAO-UN, 2018**

### 2.1.4 Food Value of Tea

Tea is a very healthy beverage that provides the body with valuable antioxidants in addition to a variety of vitamins and minerals (Chang *et al*., 2021).

Nutritional Value

One of the main benefits of tea is that it is a very low-calorie beverage. Table 2.5 shows the different composition of one (1) gram of tea. For example, 8-ounce of tea serving, devoid of sugar, milk, and honey, has just about 2 calories, 0.76g (carbohydrate), 0.02g (protein), and no fat. Compared with the regular tea, other drinks (including those containing some amount of tea) have other ingredients that increase their calories (fatsecret.com, 2022).

Vitamins and Minerals

In addition to being loaded with antioxidants, tea is equally a rich source of minerals and vitamins. Computed from an average daily consumption of 5 to 6 cups of tea, about 25% of daily recommended value for vitamin $B_2$ and 10% of daily need for zinc and iron is supplied. This amount of tea also contains 45% daily need for the mineral manganese- a mineral essential for proper bone health and development (fatsecret.com, 2022).

### 2.1.5 Benefits of green and black tea

Green tea is particularly rich in epigallocatechin-3-gallate (ECCG) and myriads of abundant antioxidants. The antioxidants present in green tea are highly health beneficial including possessing anticancer properties (breast, bladder, lung, and other types of cancer). It is also implicated in the reduction of neurodegenerative disease risk such as Parkinson's and Alzheimer's. Green tea may also help to alleviate body cholesterol levels. Black tea is a product of fermentation and has the highest caffeine content among tea types. Aside from forming the base for popular tea drinks, it is a popular lung protector, scavenging the impact of cigarette smoke on the lungs. The antioxidants found in black tea may also help to reduce risk for stroke (Hilal and Mubarak, 2013).

**Table 2.5: Nutritional composition of tea**

| Popular types of tea | Fat (g) | Carbohydrate (g) | Protein (g) | Calorie |
|---|---|---|---|---|
| **Regular tea (app. 8 floz or 1 mug)** | | | | |
| Brewed tea | – | 0.71 | – | 2 |
| Tea (no sugar) | – | 12.51 | 0.02 | 47 |
| Brewed tea (decaffeinated) | – | 0.71 | – | 2 |
| Unsweetened tea | – | 0.76 | 0.02 | 2 |
| Tea (has low-calorie sweetner) | – | 1.66 | 0.05 | 7 |
| **Instant tea (powdered)** | | | | |
| Instant tea (regular) | – | 1.09 | 0.09 | 5 |
| Instant tea (decaffeinated) | – | 0.4 | 0.14 | 2 |
| Instant tea with low calorie sweetner | – | 1.04 | 0.05 | 5 |
| Instant tea (has sugar) | – | 5.95 | 0.19 | 24 |
| Instant tea (presweetened) | 0.14 | 20.5 | 0.02 | 83 |
| **Tea with milk** | | | | |
| 1 tea cup (6 floz) | 0.84 | 1.51 | 0.95 | 17 |
| 1 mug (8 floz) | 1.11 | 2.01 | 1.26 | 23 |
| **Tea with milk and sugar** | | | | |
| 1 tea cup (6 floz) | 0.82 | 4.97 | 0.93 | 30 |
| 1 mug (8 floz) | 1.09 | 6.62 | 1.24 | 40 |
| **Herbal tea (1 mug or 8 floz)** | | | | |
| Herbal tea | – | 0.47 | – | 2 |
| Herbal tea with low calorie sweetner | – | 1.35 | 0.02 | 7 |
| Herbal tea with sugar | – | 12.32 | – | 47 |
| **Leaf tea (1 mug or 8 floz)** | | | | |
| Leaf tea | – | 0.71 | – | 2 |
| Decaffeinated leaf tea | – | 0.71 | – | 2 |
| Leaf tea with low calorie sweetner | – | 1.59 | 0.02 | 7 |
| Leaf tea with sugar | – | 12.56 | – | 47 |

**Source: https://www.fatsecret.com/calories-nutrition, (23rd April, 2022)**

### 2.1.6 Effect of fermentation on black tea fungal contamination and mycotoxin production

Mycotoxins and other food contaminants pose serious threat to human health and safety. Mould development can occur at phase of food processing including during growth, harvest, storage, and processing. Extant literature reveals that many West Africans get heavily exposed to mycotoxins through food (Hamad *et al*, 2023). According to a world development report, mycotoxin-induced diseases are implicated in life expectancy reduction in developing nations and West African countries are especially vulnerable to mycotoxigenic moulds due to their enabling environmental conditions- tropical climate with all-year-round high relative humidity and temperature (Hassane *et al*., 2017). This factor is further exacerbated by poor or inadequate facilities for processing, transportation, and storage, as well as skilled labour. Furthermore, many mycotoxin-producing fungi also frequently contaminate agricultural produce (including grains, vegetables, fruits, and seeds). Examples of relevant mycotoxigenic fungi in food include *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, and *Alternaria*. They can grow under varying environmental conditions (moisture, temperature, and pH) utilizing a wide range of substrates and as a result, infest most seeds, fruits, grains, and vegetables pre- and post-harvesting, and during storage and transportation (Hernandez and Martinez, 2018). There is a high possibility of mycotoxin-production where mould grows and these fungi are of three classes:

a. Field Fungi: known to infest produce before harvest.
b. Storage Fungi: attack commodities after harvesting and storage, and are predominantly *Penicillium spp* and *Aspergillus spp.*
c. Advanced Decay Fungi: infestation occurs after substantial decay. Examples are *Chaetomium* and *Fusarium.*

Black tea, in its case, can become infested with mycotoxigenic moulds during fermentation and drying, or during accidental re-wetting of dry leaves during storage or transportation (Hassane *et al*., 2017). Meanwhile, farmers in developing economies such

as Nigeria are lacking in the adoption of recommended technologies and methods for tea growth, harvesting, desiccation, and storage, which can substantially curb mould growth and mycotoxin production. This is because these methods are mostly costly and cannot be immediately implemented in such regions (Aroyeun *et al.*, 2011).

## 2.2 Coffee

Coffee is reportedly the globally second most-consumed beverage made by roasted coffee seeds (primarily *Coffea arabic, Coffea robusta L. Linden,* and *Coffea liberica W. Bull ex* Hiern) infusion (Galarce-Bustos *et al.*, 2014). The seed of the coffee plant is called the coffee "bean". The genus Coffea contains about 25 to 100 coffee species which can either grow as shrubs or tall trees. However, the most important and relevant species widely grown for consumption are the *Arabica* and *Robusta*. Coffee beans and berries can become contaminated and infested by different moulds during growth and processing (Galarce-Butos *et al.,* 2014). Of these mould, *Aspergillus niger*, *Aspergillus carbonarius, Aspergillus ochraceus,* and *Penicillum verrucosum* are established ochratoxin A (OTA) producers (Mitchell *et al.,* 2017). Although OTA is on the front burner, coffee contains myriads of other mycotoxins. In their study, García-Moraleja *et al.* (2015), implemented a technology that can simultaneously identify approximately 21 mycotoxins (OTA, sterigmatocystin, diacetoxyscirpenol (DAS), $AFB_1$ and $AFB_2$, enniatin (ENA), $ENA_1$, ENB, $ENB_1$, 3-acetyldeoxynivalenol (3-aDON), $AFG_1$ and $AFG_2$, NEO, T-2 toxin, beauvericins (BEA), NIV, $FB_1$ and $FB_2$, 15-acetyldeoxynivalenol (15-aDON), HT-2 toxin, and DON) in coffee beverages.

### 2.2.1 World coffee production

People consume coffee every time of the year. However, its production does not occur all year around. As a result, long-term storage is crucial for availability and quality preservation. Approximately 60 countries in the tropical and sub-tropical belt extensively cultivate coffee, with the produce serving as a major source of export for some (Banti and Abraham, 2021). Total export for coffee (in its raw and processed forms) was about 118.96 million bags in 2020/21, representing a 1.9% increase as opposed to 116.6 million bags recorded in 2019/20 (ICO, 2021), implying an increase

in coffee production, consumption, and commerce. The *Arabica Coffea* and *Coffea canephora* (Robusta) are the most widely consumed species globally. However, Arabic is considered the premium coffee and attracts equivalent prices based on its sensory characteristics. Table 2.6 depicts the foremost 10 coffee-producing countries.

### 2.2.2 History and development of coffee in Nigeria

Coffee cultivation in Nigeria is traceable to the nineteenth century (Adeleke *et al.,* 2017), although production was on a very small scale. Aderolu *et al.*, (2014), reported coffee as second most traded commodity after crude oil and this enhanced balance of trade among developed and developing nations of the world. Nigeria did fairly better in coffee production compared to other tree cash crops. In Nigeria, coffee production is primarily undertaken by subsistence farmers in the Mambilla highlands of Taraba State and lowlands in Ogun, Kogi, Abia, Ondo, and Kwara States thus, coffee was a widely traded crop, immensely contributing to the country's economy before the oil boom. More than 80% of coffee production is handled by subsistent farmers with inadequate technical exposure and skills in developing countries, especially in Nigeria, and are currently experiencing low market valuation due to poor productivity, crop management, and in many cases, farm abandonment (Adeleke *et al.,* 2017). Although the Federal Department of Agriculture (FDA) reported that coffee production began in the 1920's, archive document indicate that production could have began before then with records showing exportation at 5.5 and 25.2 tons in 1896 and 1909, respectively. The dwindling demand for indigenous cultivated species; Coffee liberica and Coffee abeokutea, paved way for the introduction of commercially important coffee species in the 1930's. William, reported that *Coffea canephora* (Robusta) and arabica *Coffea* accounted for 90% and 4% of coffee exportation (Adepoju *et al*., 2017). Nigeria has primarily invested in *C. canephora* (Robusta), although *C. arabica* is grown in Mambilla platueau some parts of Obudu cattle ranch located in Cross River state. Coffee production takes place in about 14 states in the country, spanning over 5000 hectares, with production in the following order: Robusta (94%), Arabica (4%), and Liberica (2%). As a result, Nigeria is a ripe ground for instant coffee trading. Meanwhile, Quillou and Java contribute about 15% and 8.5% of the reported robusta

cultivation. States known for coffee cultivation include Cross River, Oyo, Bauchi, Ekiti, Delta, Ogun, Jos, Akwa Ibom, Kogi, Taraba, Kwara, Ondo, Abia, and Edo (Adepoju *et al.*, 2017). Table 2.7 shows the trend in production of coffea in Nigeria between the year 2009 and 2018.

**Table 2.6 Foremost Coffee-producing Countries**

| S/N | Country | Tonnes |
|---|---|---|
| 1 | Brazil | 2,609,060 |
| 2 | Vietnam | 1,200,000 |
| 3 | Indonesia | 495,000 |
| 4 | Colombia | 468,000 |
| 5 | Ethiopia | 390,000 |
| 6 | Peru | 326,580 |
| 7 | India | 319,980 |
| 8 | Honduras | 270,000 |
| 9 | Mexico | 258,000 |
| 10 | Guatemala | 225,000 |

**Source: FAO, 2015**

**Table 2.7 Coffee Production Statistics in Nigeria**

| Year | Area harvested (ha) | Yield | Production quantity (tonnes) |
|---|---|---|---|
| 2009 | 1800 | 11333 | 2040 |
| 2010 | 1990 | 12063 | 2400 |
| 2011 | 1942 | 13001 | 2525 |
| 2012 | 1893 | 12767 | 2417 |
| 2013 | 1639 | 12811 | 2100 |
| 2014 | 1596 | 12822 | 2047 |
| 2015 | 1527 | 12766 | 1950 |
| 2016 | 1352 | 12561 | 1698 |
| 2017 | 1517 | 12513 | 1898 |
| 2018 | 1483 | 12465 | 1849 |

**Source: Alli *et al.* 2021; FAOSTAT, 2020**

### 2.2.3 Chemical composition and nutritional value of coffee

Like many other plant tissues, green coffee beans contain insoluble polysaccharides like hemi-cellulose and cellulose (50%w/w). They also have soluble carbohydrates like monosaccharides (galactose, fructose, glucose, and arabinose); oligosaccharides (sucrose, stachyose, and raffinose); and polymers of glucose, galactose, arabinose, and mannose. Soluble carbohydrates are vital components of coffee extract that function and stabilizing foam and aroma binder. They also increase viscosity and sedimentation. Furthermore, coffee contains volatile and non-volatile aliphatic acids. Volatile acids include isovaleric, acetic, butanoic, hexanoic, and propanoic acids, while non-volatile acids include quinic, malic, and citric acids. Lastly, coffee beans contain waxes and oils, representing about 8% to 18% of the total dry mass; free amino acids and proteins (9%-12% w/w); minerals (3-5% w/w) (Adepoju *et al.*, 2017; Alli *et al.*, 2021; Ghosh and Venkatachalapathy, 2014). Table 2.8, highlights the chemical constituents of green Robusta and Arabica beans.

**Table 2.8 Chemical composition of green Arabica and Robusta Coffee beans (g/100g)**

| Components | Arabica coffee | Robusta coffee |
|---|---|---|
| Polysaccharides | 49.8 | 54.4 |
| Quinic acids | 0.4 | 0.4 |
| Amino acids | 0.5 | 0.8 |
| Lipids | 16.2 | 10.0 |
| Sucrose | 8.0 | 4.0 |
| Chlorogenic acids | 6.5 | 10.0 |
| Caffeine | 1.2 | 2.2 |
| Aliphatic acids | 1.1 | 1.2 |
| Proteins | 9.8 | 9.5 |
| Minerals (as oxide ash) | 4.2 | 4.4 |
| Trigonelline | 1.0 | 0.7 |
| Water | 8-12 | 8-12 |
| Reducing sugars | 0.1 | 0.4 |
| Other sugars | 1.0 | 2.0 |
| Volatile aroma | traces | traces |

**Source: Ghosh and Venkatachalapathy, 2014**

## 2.3 Mycotoxins occurrence in beverages and foods

Mycotoxins are naturally occurring low molecular weight biochemical compounds synthesized by filamentous fungi with no apparent biochemical functions in their producers. Under favorable and enabling conditions, mycotoxins are capable of creating diseases in humans and animals (Adhikari, 2017). Identified agro-economically relevant mycotoxins include aflatoxins (Afs), fumonisins (FBs), trichothecenes (deoxynivalenol DON, T-2 toxin, nivalenol NIV, and HT-2 toxin), ochratoxin A (OTA), ergot alkaloids, zearalenone (ZEN), patulin (PAT), and tremorgenic toxins, and are mostly produced by Penicillum (OTA and PAT), Aspergillus (Afs, PAT, and OTA) and Fusarium (NIV, ZEN, HT-2, DON, and T-2). Food and beverage contamination pre-harvesting, during harvesting, drying, and in storage have been established (Aiko and Mehta, 2015). Nonetheless, some mycotoxins, especially those of ergotism, commonly occur in the field. Meanwhile, ochratoxins, aflatoxins, and fusarium toxins draw substantial attention as feed and food contaminants. OTA is implicated in the occurrence of chronic kidney disease, Balkan Endemic Nephropathy, and urinary tract tumors. As such, they have been equally categorized by the International Agency for Research on Cancer [IARC] as potential cancer-inducing compounds in group 2B (IARC, 1993). Thus, coffee (raw and processed) mycotoxin-contamination is a widely-studied area due to its tetratogenic, nephrotoxic, immunosuppressive, and hepatotoxic implications. T-2 toxin and dexoynivalenol (DON), on the other hand, are naturally occurring and possibly inevitable contaminants in specific commercial edibles and agricultural produce. Alongside tricothecenes, these toxins are chief mycotoxins in temperate regions (Kachuei, 2014). In reality, it may be impossible to have a food supply chain free of mycotoxin contamination. The technology that can eradicate all naturally occurring mycotoxin or eliminate a particular mycotoxin-type from feed and food is not available. Hence, the key to unlocking mycotoxin management is to set realistic objectives. Probiotics, natural remedies, and amino-acids are some ways to limit exposure to trichothecene toxins even as the search for an antidote continues (Adhikari *et al.,* 2017). Deoxynivalenol, trichothecene, and T-2 toxins are frequently encountered in coffee and tea derivatives. Hence, regulation and mitigation techniques should be stringent.

### 2.3.1 Occurrence of T-2 toxin

T-2 toxins are one of the most health-hazardous mycotoxins on a global scale. They are formed from the trichothecenes ring, and are part of a group of chemically-related mycotoxins produced by *Fusarium, Myrothecium,* and *Stachybotrys.* Specifically, T-2 toxins lack a carbonyl groups at on the $8^{th}$ carbon and belong to the type-A trichothecene group (Lei *et al.,* 2016). These toxins are non-volatile and can withstand harsh environmental conditions (involving temperature and light alterations), making them resilient to degradation. However, they are susceptible to strong alkalinity and acidity and can become deactivated in such media. T-2 toxins are water-insoluble but soluble in solvents such as methanol, ethyl acetate, propyl glycol, acetone, ethanol, and chloroform. They also exhibit stability under varying environmental conditions, including during autoclaving. However, T-2 inactivation can be achieved by increasing temperatures to $900^0$F for 10 minutes or extending time heating time to 30 mins at $500^0$F (Kachuei *et al.,* 2014).

### 2.3.2 Occurrence of deoxynivalenol

The Fusarium fungi, mostly found in temperate climes, are highly responsible for the synthesis of this type-B trichothecene mycotoxin named deoxynivalenol. It is globally found in many crops. Crop contamination and infestation by these fungi is usually aided by high humidity as the fungi depend on certain environmental variations to thrive (Lei *et al.,* 2016). Feeds and foods related to cereals are particularly susceptible to contamination by these fungi (Sirot *et al.,* 2013). It is often also prevalent in tropical-crop-based beverages (Garcia-Moraleja *et al.,* 2015). DON can resist temperature variations to some extent, and it is chemically stable. Like its counterparts, DON primarily bars protein synthesis. Symptoms like bloody diarrhea and vomiting are acute gastrointestinal disturbances often associated with the consumption of DON-contaminated food and feeds. However, DON can lead to anorexia, weight gain inhibition, and modified nutritional efficiency with prolonged exposure. Meanwhile, humans and animals exhibit similar acute effects with DON exposure, and the mycotoxin is linked to quite a number of human intoxication cases in Asia (IARC, 1993).

### 2.3.3 Mechanism of action of deoxynivalenol

Trichothecenes are efficient in the inhibition of DNA and RNA synthesis, as well protein production. They can also affect the cell membrane. Inhibition is initiated by binding to active ribosomes and polysomes. This step effectively disrupts the peptide linkages, resulting into a cascade reaction of initiation and termination reduction and an eventual interruption of the ribosomal cycle. Meanwhile the toxicity of the trichothecenes are derived from the 12, 13-epoxytrichothecene ring. Protein inhibition can also occur via two mechanisms: the disruption of the first step in protein synthesis (used by T-2, DAS, and HT-2) and the elimination of the elongation-termination stage (driven by DON). Due to the effectiveness in inhibition protein, RNA, and DNA synthesis, trichothecenes are a strong red alert for cells with high proliferation rate. They are also effective in the killing of eukaryotic cells, resulting into mitosis inhibition and cell breakage (Luo *et al.,* 2018).

### 2.3.4 Tolerable Daily Intake (TDI)

The Committee (TC), after fully evaluating the members of the trichothecene group, ascribed TDI to DON and temporal TDIs to T-2 toxin, nivalenol, and HT-2 toxin until a complete group assessment is done. The temporal TDI inscriptions were used for T-2 toxin and nivaleno due to incomplete data. As for DON, its full TDI is placed at 1 µg/kg bw/day; t-TDI for nivalenol is 0.7 µg/kg bw/day; while t-TDI for HT-2 toxin plus T-2 toxin is 0.06 µg/kg bw/day (European Commission, 2017). Table 2.9 show the level of deoxynivalenol contamination allowable in food raw materials intended for further production processes.

## 2.4 Reduction of mycotoxins

Several well-thought intervention strategies can be applied to control acute mycotoxin contamination in commodities (Hamad *et al.,* 2023). Such measures can be biological, chemical, or physical.

### 2.4.1 Field control

Field crops are treated with fungicides to reduce pre-harvest contamination, which is considered a traditional method of control (Munkvold, 2014). However, more environmental friendly options exist for such purposes.

#### 2.4.1.1 Breeding for disease resistance

Plant breeding describes the traditional or crude approaches to alleviating plants' resistance to fungal infection. Such methods are considered promising as it has been proven effective in curbing *Fusarium* menace in corn and wheat. It has also produced some positivity in mitigating against aflatoxin deposition or *Aspergillus flavus* infection in peanut and corn cultivars (Munkvold, 2014). Resistant and susceptible host plants cultivars are different ways, including production of constitutive and inducible kernel proteins (e.g. zeamatin) and kernel pericarp wax content (Rasheed *et al*., 2017). Molecular marker-assisted breeding techniques are increasingly being used with field crops along with traditional plant breeding technologies. Marker-assisted selection (MAS) is a technique in crop breeding that encompasses using molecular genetic markers as indirect crop selection tools. MAS functions using a quantitative trait loci (QTL) platform, which involves identifying chromosomal areas that can be selected based on a pre-defined statistical association with a measurable characteristic in an isolating population. These areas exhibit one or more genes that may not be impactful on resistance traits when considered singly (Zhao *et al.,* 2014). For instance, resistance to head blight by *Fusarium* in barley and wheat as well as aflatoxin-resistance observed in corn requires more than a few genes for proper expression, and they can be sufficiently captured in large segregating populations using QTL. Hence, molecular markers provided by QTL can provide an improved alternative to the popular traditional breeding techniques (Bhat *et al.,* 2015).

**Table 2.9 Tolerable daily intake for DON**

| Food sample | DON (µg/kg) |
|---|---|
| Baby food (cereal based) | 200 |
| Maize gruel (> 500 microns) | 750 |
| Maize gruel (≤ 500 microns) | 1250 |
| Bread, Pastries, Biscuits | 500 |
| Dry pasta | 750 |
| Flour, Bran, Germ | 750 |
| Wheat, Oat, Maize | 1250 |
| Maize for wet milling | 1750 |

**Source: C.R.N (EC), 1881/2006/2017**.

#### 2.4.1.2 Bio-competitive riddance

In a promising move called bio-competitive exclusion technique, a non-toxigenic *Aspergillus flavus* can be used to control toxigenic *Aspergillus parasiticus* and *Aspergillus flavus* in peanut plantations (Zanon *et al.,* 2016). This technique has been proven an effective biological control of peanut-aflatoxin contaminaton. In another study, sporidesmin contamination in some New Zealand pastures have been successfully managed using antagonistic strains of *Pithomyces chartarum*. Bio-competitive exclusion also shows promising results in the reduction of wheat DON content and corn fumonisin levels (Luo *et al.,* 2018). Heat treatment followed by replacement with a non-toxigenic strain have been suggested for the control of toxigenic *Acremomium lolii* in the lolitrem of rye grass. Lolitrem, a tremorgenic mycotoxin is renowned for its rye stagger induction in grazing animals. *Acremonium lolii* can also be replaced because it does not depend on lolitrem for its insect anti-feedant operations (Sipos *et al*, 2021). The incorporation of competitive antagonists into the field is another biological control method for mycotoxin-synthesizing fungi. For examples, *Fusarium graminearium,* the causal microbe of wheat *Fusarium* head blight, can be controlled using *Cryptococcus nodaensis, Bacillus subtilis,* and other *Cryptococcus* isolates. In the same vein, fumonisin concentrates in corn roots have can be successfully reduced using endophytic *Enterobactercloaceae*. Furthermore, *Aspergillus flavus* shows promising susceptibility to yeasts isolated from pistachio and almonds (Zanon *et al.,* 2016).

#### 2.4.1.3 Genetic engineering

Plant genes can be modified to improve resistance to mycotoxins or fungal infection through genetic engineering. The technology can also be used to introduce desirable traits such as detoxification into the target plant. For instance, microorganisms capable of converting fumonisins to $CO_2$ have been reported (Luo *et al.,* 2018). Genetic engineering equally enabled the identification of fumonisin esterase enzymes in transgenic corn plants. The observation revealed lower fumonisin B1 (FB1) content in kernels of corn plants than in the traditional, unmodified corn plants. Meanwhile, study on rice using genetic engineering tools also revealed the possession zearalenone detoxifying enzymes and trichothecnes genes in the plant (Wu *et al.,* 2014). After successfully identifying aflatoxin growth reduction methods in crops, Sipos *et al.* (2021), infused the isolated peptide genes

through genetic engineering into corn and cotton to combat infestation by *Aspergillus flavus.* Furthermore, fumonisins esterase enzymes which are peptides with antifungal properties isolated from transgenic crops have the potential to inhibit fungal development and mycotoxin content in crops. These antifungal peptides function by inhibiting cell wall production or disrupting cell membrane stability (Wei *et al*., 2017). Accordingly, choloroperoxidase, a bacterial enzyme has shown potential antifungal characteristics against *Aspergillus flavus* in *in-vitro* diagnostics. Meanwhile deoxynivalenol (DON) binds to the 60s subunit of ribosomal proteins (RPL3) to interfere with cell protein synthesis (Wu *et al*, 2014). An effective way to improve *Fusarium* head blight resistance in crops is to hold off binding between DON and RPL3 protein. This genetic modification reported by Bhat *et al.* (2015), discovered more DON tolerance in transgenic corn harboring modified RPL3 than their unchanged counterparts. Tobacco and wheat have also been documented to contain transgenic trichothescene resistance genes PDR5 and TRI101 (Aureli *et al.*, 2015).

### 2.4.2 Post harvest control

While agricultural and food industries aim to prevent and reduce mycotoxin contamination of field crops, it is worthy to note that *Alternaria, Fusarium, Penicillium,* and *Aspergillus* commodity contamination alongside some mycotoxin production are inevitable under certain enabling environmental conditions (Marin *et al*., 2013). As such storage conditions must be optimized to ensure that mycotoxin levels in feeds and foods remain minimal after harvesting. Some certain factors influence mycotoxin synthesis. These include microbial interactions, temperature, water levels in stored commodities, chemical preservatives addition, and gas composition of stored products. Using a holistic approach to simultaneously control these multiple factors will most likely yield better management results without necessarily applying extreme measures to one factor alone (Wang *et al.,* 2018).

#### 2.4.2.1 Chemical control method

**Ozonization**: This is a well-known method for breaking down aflatoxin contents of cottonseed meals and corns, and an efficient control method for moniliforme and DON. Ozonization has also shown considerable *in vitro* activity against zearalenone, cyclopiazonic acid, patulin, ochratoxin A, and secalonic acid (Hamad *et al*., 2023).

However, Adebo *et al.* (2021), reported that it may not be an efficient binding method for mycotoxins. Meawhile, there are other materials, such as zeolite and some clay that can effectively bind mycotoxins. However, there is a need for further efficacy testing for specific mycotoxins before being incorporated into foods and feeds.

**Sulphiting**: Sodium bisulphit can be used to treat aflatoxins $G_1$, $B_1$, and $M_1$ to create water soluble compounds with reduced mycotoxigenic properties (Adebo *et al*., 2021).

**Ammoniation**: This method has been found effective in the detoxification of cottonseed and cottonseed meal (Hamad *et al.*, 2023).

**Adsorption**: Aflatoxin absorption in host cells becomes impossible once they are attached to binders, limiting their toxicity range. While calcium aluminosilicates is an established and effective aflatoxin binder, zearalenone has shown considerable activity in binding but is not yet established via *in vivo* efficacy testing.

#### 2.4.2.2 Physical method

**Fermentation**: Aside from its purported organoleptic and nutritional impact on food, fermentation is also a considerably inexpensive and easy way to achieve food preservation. Fermentation may be executed using starter cultures, microbes found on the fermentation gadgets, or directly induced by normal flora in raw materials. *Candida krusei* and *Saccharomyces cerevisiae* are natural yeast cells that spontaneously induce food fermentation but can also be externally added as starter culture in the food and beverage industry. Meanwhile, yeasts are confirmed feed additives that have been commercially exploited for animal feeding since over a hundred years ago (Adebo *et al.*, 2021). As a result, LAB and yeast are promising mycotoxin/fungi control tools in animal feed and cereals.

Economic value can be lost in food, feed, animal, and crop production through many channels of production, processing, and distribution (Vidal *et al.*, 2013). Crop contamination is a significant economic loss channel even when prevailing environmental conditions are favorable (Bolechova *et al.*, 2015), highlighting the significant consequences of mycotoxins decontamination, prevention, and detoxification. Regardless of the selected decontamination strategy, it is crucial to ensure that such techniques meet some requisites such as mycotoxin inactivation, mycelia and fungal spores destruction to prevent new toxins formation, and preservation of food and feed nutrition, quality, and

palatability. Furthermore, physical characteristics must not be significant modified and economic evaluation of such technique must be positive and favorable (Haque *et al.*, 2020).

Several biological, physical, and physical mycotoxin-combating methods exist. While many chemical methods have considerable efficacy, they fail to meet up with the basic requirements, especially those involving preservation of the nutritional content of food and reaction product safety (Adebo *et al.*, 2021). As a result, food supplementation with anti-toxicity properties, and the use of yeasts or bacterial, non-nutritive sorbents, and modified yeasts for mycotoxin control are attracting great interest. However, these are not safety and acceptability concern-free, especially in terms of LAB/yeast fermentation in the local industry. The use of tropical spices is also being explored in recent times (Aroyeun *et al.*, 2011).

### 2.4.3 Effect of natural dietary supplements on reduction of mycotoxins

Natural compounds from plants are attracting great interest for food preservation as consumers become more food-safety conscious in the light of antibiotic resistance in pathogenic microbes and synthetic food additives abuse. Safety of consumers upon consumption of foods rich in synthetic chemical is a concern to food safety professionals. Hence, natural foods from herbs and spices are increasingly coming into market due to the presence of bioactive components serving nutritional and health benefits in them (Thanushre *et al*., 2019). The relationship between health and diet has also been established through many clinical and epidemiological observations. It has been demonstrated that people who consume higher proportions of seafood or plant foods such as vegetables and fruits also exhibit lower incidences of some cancers and cardiovascular diseases (Sirot *et al*., 2013). Thus, functional foods are going nowhere anytime soon. These food substances constitute a part of the primary diet but have extended health improvement properties. Meanwhile, neutraceuticals are food derivatives with activity against chronic disease and are sold as medicine in powders, tablets, capsules, syrups, and solutions. In Canada, neutraceuticals are considered natural health products. Hence, the primary and short-term goal of neutraceuticals and functional foods is living a disease-free life without necessarily elongating the life span. However, they may equally elongate lifespan in the long-run (Zhao *et al.*, 2016).

## 2.5 Factors influencing mould growth and mycotoxin production in tea and coffee and their products (beverages)

Pre-harvest variables that make agricultural commodities vulnerable to fungal contamination include overcrowding of plant populations, growing susceptible types, and rainfall flunctuations during produce maturation and harvest. Other factors that contribute facilitating field mould infestation include the presence of other diseases and seed damage by predatory activities (by rodents, birds, and insects). According to Munkvold (2014), delayed harvesting of ripe and matured produce, damage induced by pre-processing actions, and delayed drying are harvest and post-harvest related activities that can further enhance fungal growth. Leaving stored products with a residual water content of more than 10% for a long time in inadequate storing facilities can equally spur fungal growth and replication (Islam *et al.,* 2013). Meanwhile, inappropriate practices of combining products of varying grades to boost the quality of contaminated products, especially those involving significant number of fungal spores will lead to a cross contamination of previously fungal-free products (Okeke *et al*., 2018). Public unawareness of mycotoxins, poor and inadequate regulation, and introduction of contaminated food into the food system spurred by war, drought, and socio-economic and political issues are some variables increasing mycotoxin and fungi burden in Africa.

## 2.6 Spices

Naturally occurring substances hold a certain appeal over chemicals in the pursuit for food preservatives. Leaves, herbs, roots, seeds, spice, and barks have been identified as potential natural reservoirs of preservatives. The concept of phytonutrients provides an explanation to the association between disease prevention and increased consumption of aforementioned natural products in humans. Spices are supplementary additions to food for colour, aroma, and 33mmune. Many spices are now explored for their purported useful physiological impact (Ahmed *et al*., 2022).

Many studies have demonstrated that using spice-derived essential oils, as well as powder and aqueous extracts, effectively reduce mycotoxin-producing fungi. Some notable spices include ghafath (Rosaceae), chamomile, caraway, spearmint and basil (Labiateae), anise, qyssum (Compositae), hazanbul, fennel (Umbelliferae), marigold, and cinnamon

(Lauraceae) and *Aframomun danielli* (Zingiberaceae) (Adewole *et al.,* 2013; Ahmed *et al*., 2022). The aforementioned spices and many others have been proven to have a broad spectrum of activity against fungi and other pathogenic microorganisms. It is encouraging to observe that plant products are becoming research interests as opposed to synthetic compounds for food additives as consumers show more awareness and concerns about genetically modified organisms (GMOs) and synthetic compounds (Aroyeun *et al.,* 2011). Of a truth, the interest of pharmaceutical firms is turning to the utilization of natural biological compounds to alleviate human diseases originating from pathogenic microorganisms (Afzal *et al.,* 2013).

### 2.6.1 *Curcuma longa*

*Curcuma longa,* commonly known as turmeric, has been widely explored as food seasoning based on its therapeutic characteristics and piquancy, and is a member of the Zingiberaceae family. Turmeric is a renowned substance with a wide range of medicinal properties including anti-diabetic, anti-mutagenic, anti-fungal, antioxidant, antibacterial, anti-carcinogenic and anti-fertility (Afzal *et al.,* 2013). It is popularly acknowledged as the "spice of life" and the "golden spice". In India, it has found sacredness as a medicinal and potent plant (Labban, 2014). The genus *Curcuma* is a favored one with many members containing diverse beneficial biologically active compounds such as sesquiterpenes, monoterpenes, and diphenylheptanoids. Thus, the pharmacological characteristics of curcumin and related substances have been of research interest. In this study, the antimicrobial activities of *Curcuma longa* was surmised that it is potent against some bacteria including, *Escherichia coli, Staphlococcus aureys,* and *Bacillus subtilis* based on its phenolic contents like curcuminoids (Labban, 2014). The antimicrobial properties of turmeric can be traced to constituents, such as curcumin, essential oil, veleric acic, turmerol, and alkaloid. Testing for antimicrobial characteristics, turmeric-derived oil exhibited potency against seven fungi known to modify stored agricultural products. Antifungal properties (static and not cidal) were exhibited against *Penicillium digitatum, Aspergillus parasiticus, Aspergillus flavus,* and *Fusarium moniliforme* (Afzal *et al.,* 2013). In another observation, turmeric gave off a broad antibacterial spectrum of activity

against *Escherichia coli, Bacillus cereus, Bacillus coagulans, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Bacillus subtilis* (Aroyeun *et al.,* 2011).

Phytochemically, turmeric is made up of mainly of minerals (69.4%), moisture (13.1%), carbohydrates (6.3%), and protein (5.1%). Its essential oil component, extracted using steam distillation of the plant rhizomes contains sesquiterpenes (53%), curcumin (diferuloylmethane) (34%), zingiberene (25%), a-phellandrene (1%), sabinene (0.6%), and borneol (0.5%). The plant contains non-volatile and volatile compounds, too. Curlone, zingiberen, and turmerone account for its volatile compounds while curcuminoids are the primary non-volatile compounds (Taoheed *et al.,* 2017).

### 2.6.2 *Xylopia aethiopica*

*Xylopia aethiopica* is a perennial plant, with foliage all-year-round, growing up to 0.6-0.7m in diameter and 0.2m in height, with partially stripped and smooth bark, and straight stem. The plant is a rich source of xylopic and diterpernic acids, annonacine (alkaloid), and volatile aromatic oils. It also contains other compounds, including saturated and unsaturated fatty acids, limonene, α and β pinenes, proteins, 1,8, cineole, lipids, p,cymene, sesquiterpenoids, carbohydrates, linalool, and myriene (Saroj *et al.,* 2020). The *Xylopia* fruit is similar to a combination of nutmeg and cubed pepper in its taste and smell; bitter, pungent, and aromatic. In appearance, they have similar shapes to twisted bean-pods with cylindrical and dark-brown bodies. They can be 4-6cm thick and range between 2.5cm to 5cm in length. It is possible to visualize the fruit's contours from the outside with each pod containing about 5-8 kidney-shaped beans of about 5mm long (Iweala *et al.,* 2015). *Xylopia aethiopica* is a well-known spice with medicinal uses in West Africa due to its complex chemical constituents. Based on its chemical components, it has shown potential antifungal and antibacterial activities in different forms (dried and fresh fruits, root and stem bark essential oils, and leaf). It has also been used in therapy for cough, dyspepsia, and fever. In the food industry, *Xylopia aethiopica* has found utility as spice and flavor, and is a popular fragrance for cosmetics. Meanwhile, Soraj *et al.* (2020), reported that its oil can serve as a mosquito repellant. It is rich in sesquiterpenes and mono-hydrocarbons, which exhibit antioxidative properties. The tree is prevalent in West Africa, East Africa

(Senegal to Sudan) up to Angola in South Africa. It is a primary component of pepper soup in Africa.

### 2.6.3 *Piper guineense*

*Piper guineense* is a pepper of West African origin that can be used to make spices through its dried fruit. It goes by different names such as False cubeb, West African Pepper, Uziza pepper, Benin pepper, guinea pepper, Guinea cubeb, Ashanti pepper, and has been locally described as Sorowisa, Kukaube, Sasema, Kale, and Masoro (Ekpo *et al.*, 2013). It is a permanent tree climber with woody structure that can attain at least 10m in height. It has an alternate leaf system with 2-5cm long simple petiole. As a primarily tropical tree, *Piper guineense* requires evenly distributed heavy rainfall and temperature to grow well. It equally requires a rich humus soil with well-drained alluvium. However, it will also do well in a red laterite soil. Aside from the fruits, the leaves are also used in African soups as vegetable pepper. The fruits and leaves can also serve as flavors in several foods. Locals also believe that *Piper guineense* has medicinal attributes. Hence, it is sometimes added as spice to nursing mothers and pregnant women's cuisines as it is believed to aid uterine contraction (Ejele *et al.,* 2013). Many African countries and nations in the developing worlds, including Nigeria, believe and use traditional medicine, almost exclusively, to treat wide varieties of diseases like infertility, malaria, dysentery, epilepsy, and convulsion. To prepare such local medicines, seeds, barks, roots, and leaves extracts are immersed in local gin for some days. In many cases, *Piper guineense* forms part of the formulation given to nursing mothers to restore the womb to normalcy after delivery (Ejele *et al.,* 2013). In other instances, the leaves, stems, and in some cases, the stems, are used in making soups, adding a pungent and spicy aroma, and "heat" to food. It has also been documented to have bactericidal and bacteriostatic effects on susceptible bacteria. The leaves are used in treating rheumatism, bronchitis, intestinal disease, cough, and in managing infertility in women, while the fruits further serve as aphrodisiac (Ekpo *et al.*, 2013).

## 2.7 Terpenes

Terpenes encompass various hydrocarbons produced by many plant types, especially coniferous plants. Based on the number of their five carbon isoprene units, terpenes belong to any of the following catergories: carotenoids (40), triterpenes (C30), sesterterpines (C25), diterpenes (C20), sesquiterpenes (C15), monoterpenes (C10), and hemiterpenes (C5). They are found in many essential oils and flowers as the primary compounds, and have found utility as perfume fragrances and local medicine (Ullah and Khan, 2020). With a basic formula of $(C_5H_8)_n$, terpeniods may be dehydrogenated, hydrogenated, or oxygenated. There exists more than 40,000 identified terpenoids possessing various biological properties and molecular structures. With approximately 20,000 phytochemicals having established therapeutic and chemopreventive impact on cell indicator and cancer, the triterpenoids are easily the largest categories of terpenoids known (Labban, 2014).

### 2.7.1 Terpenoids in *Curcuma longa* spice

With research, it is proven that observed therapeutic activities of Curcuma species are based on curcumin and other curcumoids present in this group. Nevertheless, co-existing terpenoids, most notably bisabola and guaiane sesquiterpenoids in this group also have vital roles to play in the expression of certain biological properties in the Curcuma species. This phenomenon is illustrated in the co-occurrence of bisacurcumin, a bisabola sesquiterperniod, with curcumin. It has also been established in many studies that curcumin activity does not occur alone but as a synergy between curcumin and many notable antioxidants like coumarin, dehydrozingerone, chromanol, vitamin A & E, and flavonoids (Afzal *et al.*, 2013). In the same vein, terpenoids in curcumin do not act alone in most cases, but as a synergy of terpenoids and curcumoids. It is of interest to note that this purported synergy between the two classes of biologically active compounds may be the backbone of research interest in *C. longa* spice.

### 2.7.2 Terpeniods in *Xylopia aethiopica* spice

According to Yun-Jie *et al.* (2013), the β –Ylangene is a sesquiterpeniod with established anti-inflammatory and cytotoxic properties explored by traditional medicine. The Germacrene D compound, with a chemical name 1,6- Cyclodecadiene, 1-methyl-5-

methylene-8-(1-methylene)- ,[s-(E,E)]-, is conglomeration of volatile organic hydrocarbons that act as precursor molecules for several sesquiterpenes including selinenes and cadinenes. Other biologically active sesquiterpenoids include, trans-z-α-Bisabolene epoxide (Srinivassan *et al.*, 2014), Manoyloxide, a diterpene (Hutschenreuther *et al.*, 2010), Cis – 4,7,10,13,16,19-Docosahexanoic acid, Androstan-17-one and 3-ethyl-3-hydroxy-(5α) (Iweala *et al.*, 2015). Antioxidants such as palmitic acid, linoelic acid were also reported.

### 2.7.3 Terpenoids in *Piper guineense* spice

*Piper guineense* seeds contain varieties of useful chemicals. For example, linalool, a natural terpene alcohol has found use as a fragrance ingredient and as a flavor (NCBI, 2018). Humulene, in its case, possesses systemic and topical anti-inflammatory characteristics (Joshua *et al.*, 2016). Caryophyllene, Methyl Tetradecanoate, and m-Anisic acid are all used as flavors. Methyl stearate serves as a fermentation and antifoaming compound. Stearic acid, otherwise called octadecanoic acid, finds uses as a softening agent and surfactant. It has also been observed to reduce LDL cholesterol than other similar saturated fatty acids during clinical and epidemiologic studies. Other terpenoids with significant uses extracted from *Piper guineense* include oleic acid, (E)-β-farnesene, myristin, piperine, germacrene D, fumaric acid, oleic acid, linalool, caryophyllene, and curcumin (Ashokkumar and Ramaswamy, 2014).

## 2.8 Mycotoxins in food

Greek words ‘mykes’ and ‘toxicum’ meaning ‘fungus’ and ‘poison’, respectively form the word ‘mycotoxin’. They are secondary metabolic products derived from saprophytic fungi such as *Penicillium*, *Aspergillus*, and *Fusarium*, and have low molecular weights. The knowledge of mycotoxins is not new and goes as far back as 1800s in the incidences of “Alimentary Toxic Aleukia’ by T-2 toxins in Russia during the World War II and “St. Anthony’s Fire” induced by ergot alkaloids (Galarce-Bustos *et al.*, 2014). Animal feed contamination by these toxins globally poses serious threats to farmers and can lead to severe animal diseases and poor production results. It can also lead to significant profit losses as animals may refuse feed, have low body weight, have impaired feed conversion,

become immune-suppressed, and interfere with residues and capacities of animal products (Zhao *et al.,* 2014). Of significant economic importance in food and feed contamination are ochratoxin A, aflatoxins, and patulin (primarily synthesized by *Penicillum* and *Aspergillus* species) and trichothescenes, fumonisins, and zearalenone (referred to as *Fusarium* toxins). Food and feed contamination by these toxins can occur pre-harvest (on field), post-harvest (storage), and during processing.

As fungi can grow indiscriminately anywhere and under diverse environmental conditions, food crops remain susceptible to their contamination pre- and post-harvesting. Air-borne infestation, transmission by insect vectors, and kernel exposure after physical damage are ways to facilitate fungi contamination on the field. Other stressors such delayed harvesting, flooding, insect infestation, and drought are contamination-facilitating factors. After harvesting, inadequate facilities with poor storage conditions of warm humid conditions and poor drying further enhance production contamination by moulds. Demographic characteristics, storing time, and processing techniques are some factors that equally influence contamination rate. Without an iota of doubt, environmental factors such as moisture content, pH and temperature variation, crop densities, and exposure to physical damage exert considerable influences on food and feed fungal contamination. In their study, Zu *et al.* (2021), observed milled and rice paddy were contaminated by aflatoxin $B_1$ and *Aspergillus*. Some other studies corroborated the observation by reporting similar results in rice grain samples from United Arab Emirates, Nigeria, and Chins (Makun *et al.,* 2007). Fumonisins, ochratoxins, and aflatoxins have been identified from market-ready processed foods. Human exposure to mycotoxin often lead to chronic and acute toxicities, inflicting damage on the gastro-intestinal tract, central nervous system, the liver, and the pulmonary and cardiovascular systems (Haque *et al*., 2020; IARC 1993).

### 2.8.1 Aflatoxin

Aflatoxin $B_1$ is a well-established cancer-causing agent in humans. According to the World Health Organization, long-term aflatoxicosis has been implicated in the occurrence of hepatocellular carcinoma (HCC), the third-most cause of global cancer-related death (Zanon *et al*, 2016). After activation by the cytochrome P450, aflatoxin $B_1$ forms aflatoxin $B_1$-8,9-epoxide, the basal agent for aflatoxin $B_1$-related mutagenic properties. After

formation, the aflatoxin $B_1$-8,9-epoxide binds particularly to guanine in RNA and DNA through it N7 position, resulting to a new compound, aflatoxin $B_1$-N7-guanineadduct. Thus, aflatoxin $B_1$ suppresses the immune system and causes tetratogenic and hormonal defects by disrupting an essential step in RNA, DNA, and protein synthesis (Mokhtarian *et al*., 2020).

### 2.8.2 Ochratoxin

At high concentration, ochratoxin can damage the liver of all animal types. However, its primary site of action is the kidney, and this toxicity has been ascribed to the hindering of phenylalanine tRNA synthetase. It is also a proven carcinogen, teratogen, and immune suppressor (Mitchell *et al*., 2017) and has a documented role in causing Balkan endemic nephropathy. Research has also revealed the possible formation of ochratosxin A-DNA complex in the bladder and kidney of some cancer surgery patients in Bulgaria (Marin *et al.,* 2013).

### 2.8.3 Fumonisin

Fumonisin B is a type of mycotoxin isolated from *Fusarium moniliforme* in 1988. It is the global causal agent of ear and stalk rot in corn but can also be asymptomatic in the crop as it is an endophyte. Fumonisins can also be found in cereals such as sorghum and stored corn grain (Munkvold, 2014; Sharma and Partial, 2021). Fumonisin $B_1$ and $B_2$ can also lead to leuko-encephalomalacia in horses as well as hydrothorax and pulmonary edema in pigs (Lopez *et al*., 2018). It also hinders ceramide kinase, leading to the disruption of sphingolipd metabolism. Meanwhile, high esophageal cancer prevalence in Italy, China, and South Africa has been linked with fumonisin $B_1$ (Morcia *et al.,* 2016). According to Wang *et al.* (2014), fumonisim $B_1$ increases and decreases cyclin D1 and cyclin E expression, respectively by stimulating the replication of epithelial cells in the esophagus.

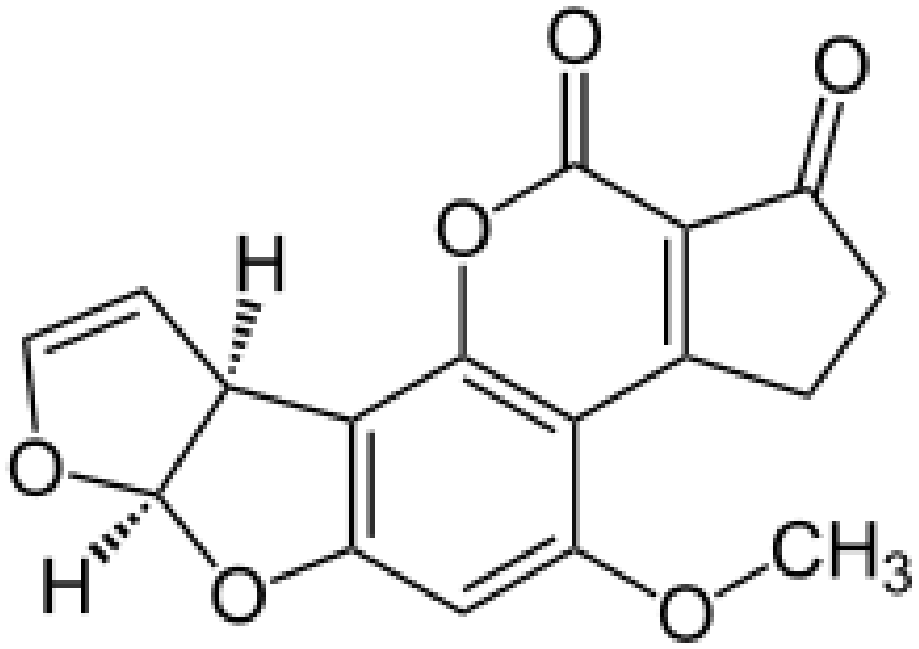


**Figure 2.1 Chemical structure of Aflatoxin $B_1$**

### 2.8.4 Zearalenone

Zearalenone, classified as a phyto-estrogen or myco-estrogen, primarily affects pigs, leading to hyper-estrogenism. Although its mechanism of action is unknown, it is hypothesized that zearalenone forms a complex with glucoronic acid after creating active forms α- and β-zearalenol (Sirot *et al*., 2013).

### 2.8.5 Trichothecenes

These are sesquiterpenoids synthesized by many fungi including *Gliocladiun, Fusarium, Stachybotrys*, *Myrothecium*, *Trichothecium*, *Trichoderma,* and *Acremonium. Fusarium spp* capable of synthesizing trichothecenes, *Fusarium poae*, and *Fusarium sporotrichoides*, are implicated in alimentary toxic aleukia outbreak in Russia at the start of the 20$^{th}$ century; in bean hull toxicoses (*Fusarium solani)* in Japan; and in akakabiobyo (red mould disease) by *Fusarium graminearum* (*Fusarium roseum*) equally in Japan (Vidal *et al.,* 2013; Wei *et al.,* 2017). The most prevalent trichothecenes are T-2, diacetoxyscirpenol, and deoxynivalenol. They are proven to induce immune-suppression, vomiting, nausea, and diarrhea in animals. They effectively hinder protein synthesis by directly stopping the actions of the peptidyl transferase in the large sub-unit of ribosomes (Wei *et al*., 2017).

### 2.8.6 Occurrence of aflatoxin-producing organisms

Mycotoxicoses by some genera *Stachybotrys, Aspergillus, Fusarium,* and *Penicillium* can result in serious sicknesses and in some instances, death. For *Penicillium* and *Aspergillus,* toxin production mostly takes place in commercially processed feeds and foods, and in stored hays and feeds. However, they mostly infest seeds while in the field. According to Hussein and Brassel (Zanon *et al*., 2016), *Rhizopus, Penicillium, Aspergillus,* and *Mucor,* initially infect crops post-harvest and sprout when they are stored. Aflatoxin has a well-established reputation among other mycotoxins. Its name was derived from the fungus *Aspergillus flavus* as it was originally isolated from it (Marin *et al.,* 2013). However, other members of the genus have been found to produce aflatoxins. Aflatoxin $B_1$, the most carcinogenic, prevalent, and toxic form of aflatoxin known, is primarily synthesized by *Aspergillus terrus.* However, other species such as *Aspergillus oryzae* and *Aspergillus flavus* can also produce it. Table 2.10 show the set limit for different mycotoxins in

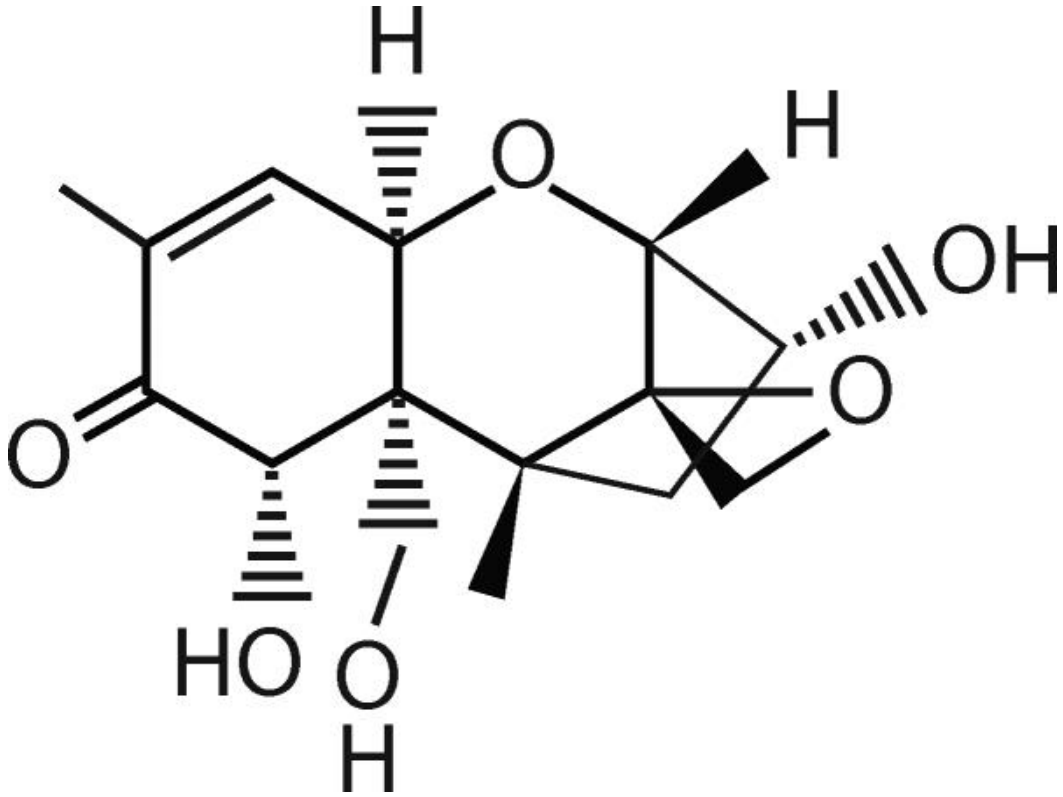


**Figure 2.2 Chemical structure of deoxynivalenol (DON).**

human food while Table 2.11 indicates the permissible limit set for Aflatoxin $B_1$ in any food material by various countries.

### 2.8.7 Methods of mycotoxins analysis

Chromatography is a separation technique involving component separation by transferring mass between a mobile and stationary medium, as well as a liquid mobile phase. High performance liquid chromatography (HPLC) is one type of chromatography and one of the most used interpretative techniques (Ahbay, 2021). Aiko and Mehta, (2015) reported different methods validated for the isolation, quantitation, and identification of mycotoxins as the following: Supercritical Fluid Chromatography-Mass Spectrometry (SFC-MS), Thin-layer chromatography (TLC), Gas-Liquid Chromatography (G-LC), Gas Chromatography-Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC), Thermospray Liquid Chromatography-Mass Spectrometry (FIPLC-MS), and Gas Chromatography (GC). However, many bio-analytic laboratories prefer the liquid chromatography. Some technological innovations for fast liquid chromatographic separation are ultra-high performance liquid chromatography (UHPLC), monolith technology, high temperature liquid chromatography, and fused core columns. However the ultra-high performance liquid chromatography (UHPLC) has become a choice technique for fast and sensitive biological and analytical quantifications (Kachuei *et al*., 2014). Some of the identified benefits of high –throughput HPLC include its amount of output relative to input and the reduction in the cost of analysis. Because it utilizes a short column length, HPLC has reduced turn-around time of analysis. As the particle size of the packed column decreases, the proficiency and resolution capacity of the HPLC increases. Liquid Chromatography-Mass Spectrometry (LCMS), on the other hand, is based on the principle of ionization to give quite a sensitive detection technology. It combines ion intensity and mass-to-charge ratios to separate ions existing in vacuum (Malachova *et al.*, 2014). In addition, mass spectrometry adds a measure of sensitivity, specificity, and a means of concurrent analysis of multiple components. As a result, LCMS integrates outstanding qualitative abilities of mass spectrometry and separation resolution of liquid chromatography. Aside from LCMS, other analytical instruments and ionization techniques exist for chemical analyses which are the following: gas chromatography mass spectrometry (GCMS), Matrix-Assisted Laser

Desorption/Ionization (MALDI-MS), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), and Electron Ionization (EI). GCMS is very efficient in the separation, qualification, and quantification of compounds. However, it is used primarily for volatile substance or gases with low molecular masses and highly heat stable substances. For LCMS, its possible use for several compounds is a singular distinguishing benefit. As long the substance is dissolvable in a mobile phase, LCSM, unlike GCSM, will analyze the most thermally unstable and in-volatile substances. As a result, LCMS is preferred for the analyses (quantitative and qualitative) of popular pollutants like personal care and pharmaceutical substance, bacteria, and pesticides) as well as emerging trace contaminants. Aside from such determination, LCMS is also used in quality control for food development and safety for products such as food additives, food supplements and organic foods, residual veterinary drugs, and mycotoxin in foods with low moisture (Parmar, 2021).

## 2.9 Criteria for decontamination of mycotoxins

It has been established that it is impossible to totally prevent or eliminate fungi contamination. Therefore, several strategies for reducing and detoxifying these toxins were reported by researchers. Whichever decontamination strategy employed, certain basic criteria must be met (Adhikari *et al.*, 2017). These methods either breakdown mycotoxins completely or mitigate the toxin level to a safe threshold. Presence of aflatoxin $B_1$ in feedstuffs can be degraded using ammonia; still, this reaction leads to the production of aflatoxin $D_1$ a product which is arguably adjudged to possess some toxicity. Again, toxic products of enzymatic degradation and its undesirable effects while employing non-native micro-organisms during fermentation, has attracted queries regarding the quality and safety of such fermented foods (Aroyeun *et al.*, 2011), and this must be laid to rest by offering the locals, innovations from their own products. It is therefore important to develop a safe and suitable detoxification strategy which does not compromise the nutritional value of such foods, hence rigorous and intense research efforts must be directed towards mycotoxins management in foods and food products.

**Table 2.10: Regulation for mycotoxins in human food (µg/kg)**

| Mycotoxins | European Union | US FDA |
|---|---|---|
| Aflatoxin $B_1$ | 2 − 8 | 20 |
| Aflatoxin $M_1$ | 0.05 | 0.5 |
| Deoxynivalenol | 2 - 7 | 1.0 |
| Fumonisins ($FB_1$, $FB_2$, $FB_3$) | 200 - 1000 | 2000 - 4000 |
| Ochratoxin | 3 − 10 | − |
| Patulin | 10 − 50 | − |
| Zearalenone | 20 - 200 | − |

**Source: Aiko and Mehta (2015).**

**Table 2.11: Allowable limits for Aflatoxin $B_1$ in foods set by various countries**

| Country | Aflatoxin $B_1$ (µg/kg) | Products |
|---|---|---|
| Argentina | 0 | Groundnuts, maize and products |
| Brazil | 15 | All foodstuffs |
| China | 10 | Rice and edible oils |
| Czech Republic | 5 | All foods |
| Hungary | 5 | All foods |
| India | 30 | All foods |
| Japan | 10 | All foods |
| Nigeria | 20 | All foods |
| Poland | 0 | All foods |
| South Africa | 5 | All foods |
| Zimbabwe | 5 | Foods |

**Source: Aiko and Mehta (2015).**

# CHAPTER THREE

# MATERIAL AND METHODS

**3.1 Study Location**: Mambilla, with coordinates 11$^{o}$.717$^{l}$ E and 7$^{o}$.333$^{l}$ N, a major tea and coffee producing area in Taraba State, Nigeria was selected. Also, samples were collected from Ibadan (coordinates 595574$^{o}$.508$^{l}$ E and 79800$^{o}$.522$^{l}$ N).

## 3.2 Materials

Mycotoxin standards ($AfB_1$, DON, Flavoglaucin, Fumigaclavin, Endocrotin, Emodin, e.t.c) and HPLC grade solvents (methanol, acetonitrile, e.t.c) were purchased from Sigma and Aldrich Chemical Company, (St. Louis MO, USA). Spectrometer, Fourier Transform Infra Red (FTIR), Spectrum BX, Perkin Elmer. NMReady-60 PRO (Alberta, Canada). Analytical balance supplied by Gibertini Electtronica, Milanese (MI), Italia. Memmert incubator (ProfiLab24, GmbH, Berlin). Agar, de-Man, Rogosa and Sharpe (MRS), was sourced from Oxoid chemicals (Hampshire, RG24 8PW, UK). HPLC grade distilled water as produced by Nanopure 11 (Barnstead/Thermolyne Corp, Dubuque, USA). DNA extraction kit was obtained from Omega Bio-Tek Inc. (Norcross, USA). Eberbach gyratory shaker (E7000, light duty, USA) and Vortex mixer-110 volt (ULAB, USA) were sourced as indicated. 250 mL Erlenmeyer flasks with number six (6) Neoprene stoppers, 16 x 125 mm culture tubes, 15 x 85 mm culture tubes, MycoSep 225 clean-up column, MultiSep 216 clean-up column (GmbH, Getzersdorf, Austria), filter paper grade 615, 0.45 µm syringe filters obtained from Millipore (Bedford, MA, USA), Amber autosampler vials with self-sealing Teflon caps (Postnova Analytics GmbH, Germany), salinized 30 mL amber glass bottles. 500 mL capacity flat bottom glass wares, aluminium foil, cotton wool, ethanol (analar), eppendof tubes, and disposable hand gloves were purchase from reliable supplier.

## 3.3 Collection of samples

A total of twenty-eight (28) samples, fourteen each were obtained from Mambilla and Ibadan respectively. These include twenty (20) tea samples and eight (8) coffee samples. The samples were coded and labeled.

### 3.3.1 Spice extraction

#### 3.3.1.1 Processing of tumeric powder

*Curcuma longa,* (yellow specie), rhizomes were washed with running water to shrug off soil and ensure it was properly cleaned. These were diced to increase the surface area, and enhance quick drying of the rhizomes, sun-dried and packed in a clean air-tight bag. Dried turmeric were crushed and subsequently fine-milled with the aid of Veronica Expert commercial mixer grinder 2JARS, 1.6HP (India). Powdered turmeric was screened (0.25mm), packed in air-tight bag and kept until use on the shelf at room temperature (27 ± 2 °C), (Labban, 2014).

#### 3.3.1.2 Processing of *Xylopia aethiopica* powder

Dried *Xylopia aethiopica* fruits were toasted at 110 ± 10 °C to free the seeds, cleaned and milled into powder with the aid of a hammer mill laced with 0.25mm mesh. This method was reported by Ugwuona (2014), but with slight modification. The obtained powder was packed in air-tight bags and stored at 27 ± 2°C).

#### 3.3.1.3 Processing of *Piper guineense* powder

Dried fruit of *Piper guineense*, were air dried at room temperature for 3 days. Dirt, immature fruits with other extraneous materials were taken off. This was milled to powder using a Veronica Expert commercial mixer grinder 2JARS, 1.6HP (India), screened with a 0.25 mesh, packed in air-tight container and kept/stored at 27 ± 2°C (Okeke *et al*., 2018).

## 3.4 Production of tea and coffee

### 3.4.1 Production of green tea and black tea

Fresh leaves, a bud and two, were picked, ‘fine plucked’ manually from tea plant (*Camellia sinensis*). These leaves were processed to green tea in line with the conventional method (Singh *et al*., 2014) employed by processors (see Figure 3.1).

About 5000 g were washed with clean water from running tap. The leaves were placed in hot pan at 115$^{o}$C and turned for about 8 to 10 minutes, thereafter packed in a basket and allowed to cool for about 5 minutes at room temperature. Rolling was done with the aid of rolling machine (YX-6CRT-25B, China), to disrupt the leaf texture, squeeze out the tea juice, improve the taste, benefit the stripe coiling, drying and shaping. The furled leaves were laid out on trays/drying mats and ventilated till the product became crispy. Upon completion of drying, the rolled leaves were coarsely milled with the aid of a manual plate corona mill No 8 to obtain a coarse product which was sieved using a USA standard testing sieve No. 30. The product was packed in an air tight container and stored at room temperature. Figure 3.2 describes the processes involved in the production of black tea. *Camellia sinensis* leaves (5000 g) were washed with tap water and allowed to drain in a basket. The drained leaves were thinly spread on drying mat laid out under shade and allowed to wither for 12 hour. To ensure the leaves were pliable for rolling, they were allowed to lose about 60 % of their moisture by constantly turning and weighing at an interval of about 30 min. The withered leaves were rolled using a manual corona plate mill. Rolling enhances disruption of the leaf cells and exposed some of the essential oils and juice necessary for oxidation to occur. The leaves were heaped, allowed to stand for 3 hours and thereafter, freely spread-out on mats and left at room temperature for 3 hour. Darkening of the leave colour was observed resulting from fermentation/oxidation processes. The fermented leaves were sun-dried till the moisture level was reduced to 6 % or 7 %. Coarse milling was done using a manual corona plate mill and sieved using a USA standard testing sieve No. 30 and packed into an air tight container.

### 3.4.2 Production of green coffee and black coffee

Green coffee was produced as described by Banti and Abraham (2021), but slightly modified as shown in Figure 3.3. Matured ripened coffee cherries were plucked and about 5000 g was cleaned of extraneous materials. This was spread out on a clean slab and allowed to stand under sunshine till dried. The shells were opened; the coffee collected and further dried till very low moisture content was achieved. The dry coffee was stored in an air-tight zip-lock bag and milled when needed for analysis.

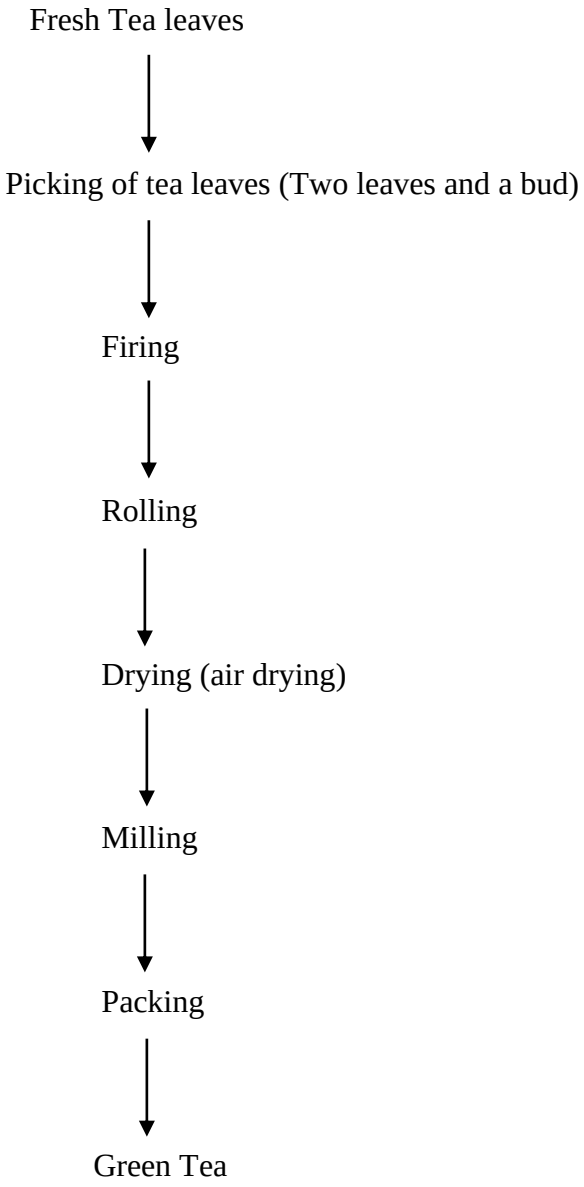


**Figure 3.1:** **Production of green tea** (Singh *et al*., 2014).

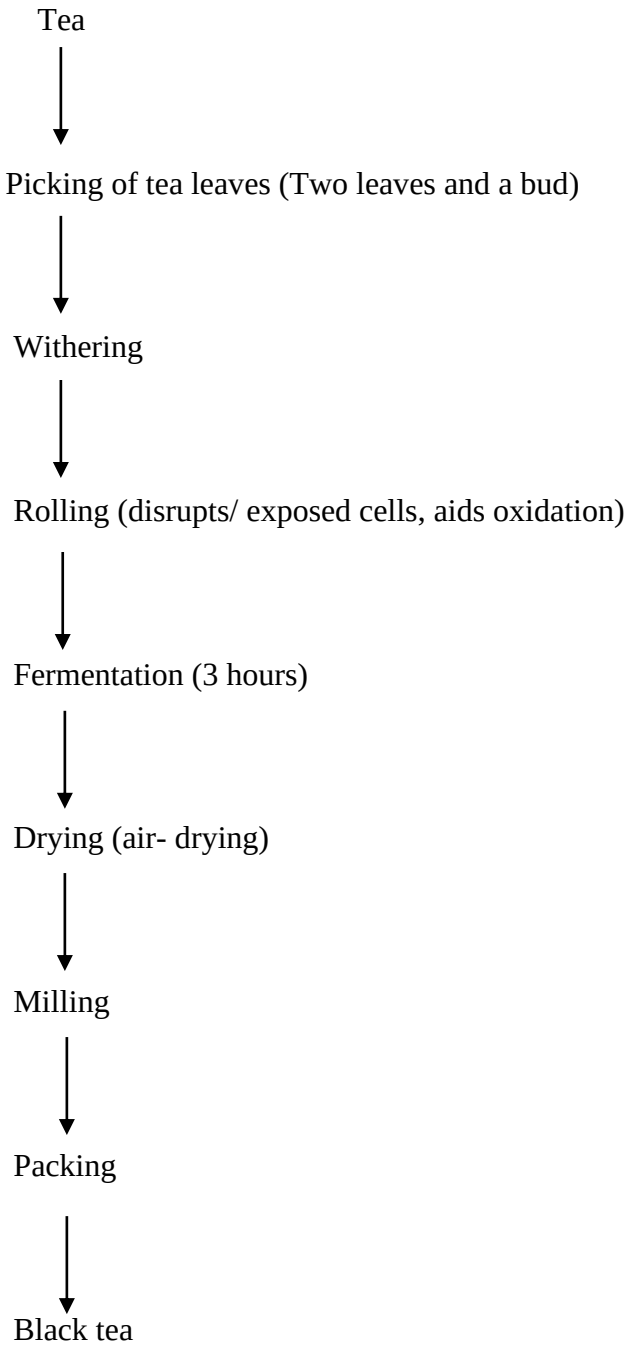


**Figure 3.2** **Production of black tea (**Singh *et al*., 2014).

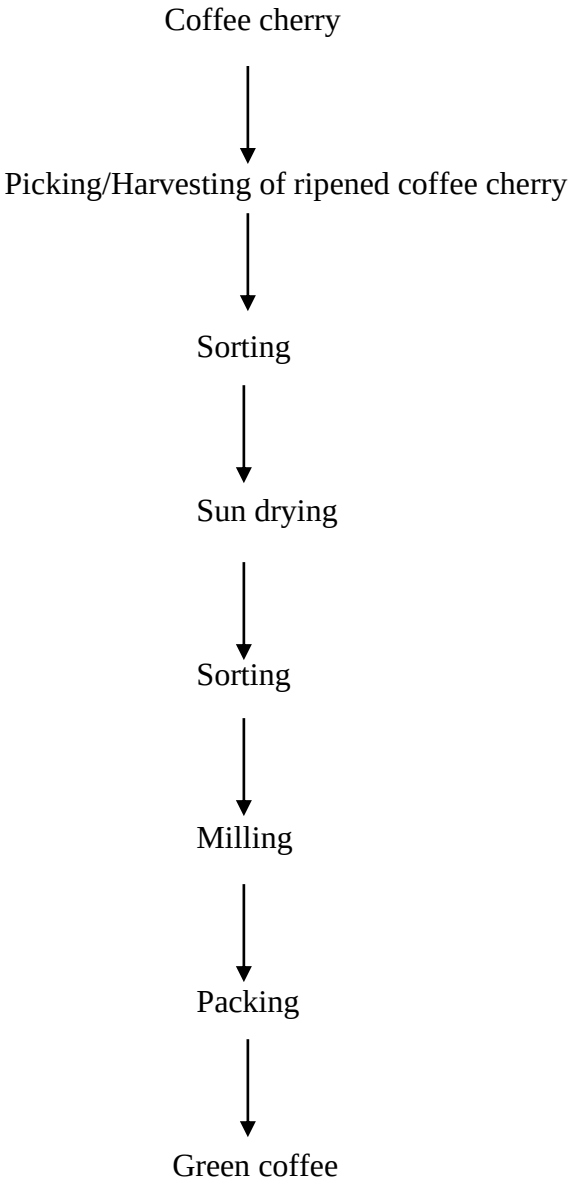


**Figure 3.3** **Production of green coffee** (Banti and Abraham, 2021).

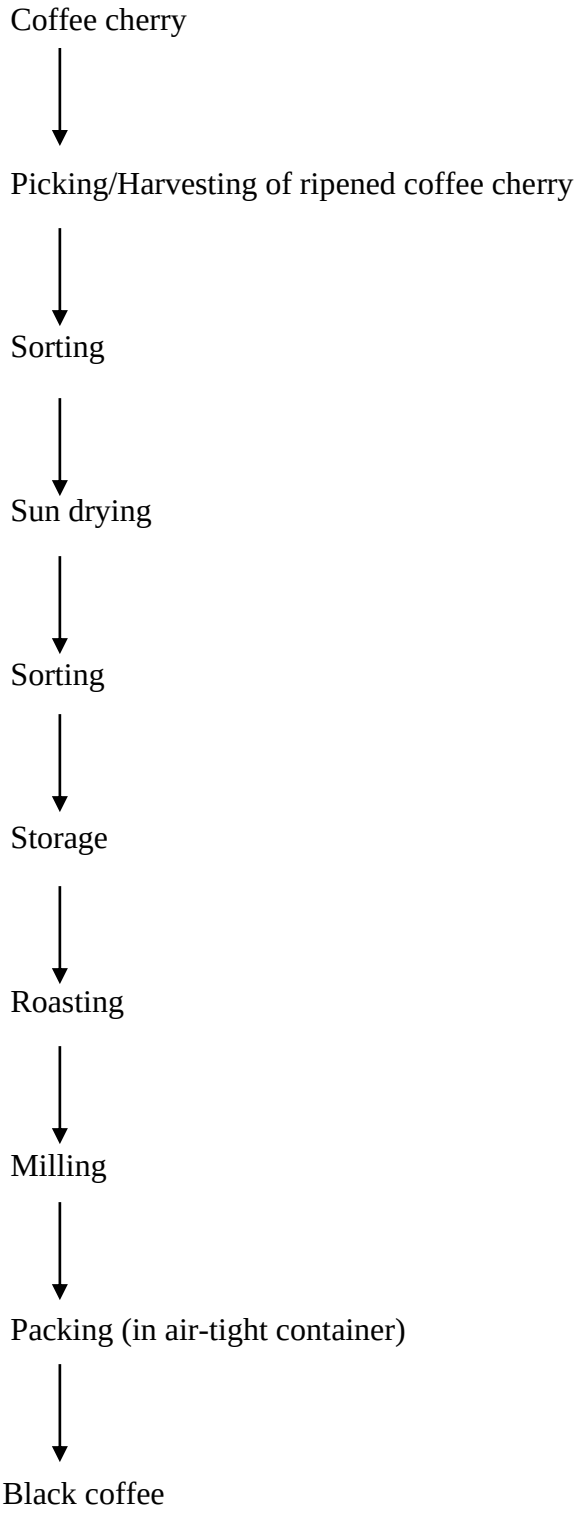


**Figure 3.4** **Production of black coffee** (Banti and Abraham, 2021).

Black coffee (see Figure 3.4) was processed by obtaining matured, ripened coffee cherries and cleaned by removing dirt (Banti and Abraham, 2021) with modification. These were spread out on clean surface and allowed to stand in sunshine for twenty days. These were break- open and coffee collected. The moisture level of the coffee had reduced to 7 %. The coffee were roasted at a temperature of 200 $^{o}$C for 25 minute and packaged in an air-tight zip-lock bag pending usage. This was milled when needed.

## 3.5 Experimental plan

The Tea and Coffee were stored at different conditions for six months (March to August). A batch was held at ambient condition; room temperature (28±3 $^{o}$C), relative humidity (70±5 %), and the other batch, held at modified condition; temperature (15±5 $^{o}$C), relative humidity of fourty to fifty percent (45±5 %). Thereafter, screening for the presence of fungi and mycotoxin metabolites were done (AOAC, 2012). Toxins level in tea and coffee were quantified. Approximately 1000 µg/g, 2000 µg/g 3000 µg/g and 4000 µg/g crude powder of *Curcuma longa*, *Xylopia aethiopica* and *Piper guineense* were added to tea contaminated with mycotoxins and stored for another six months. The effectiveness of these spices at reducing fungi metabolites in bagged tea was evaluated at the end of six months period. Moreover, response of fungi genera capable of producing $AfB_1$ and DON under conditions such as; available water, $a_w$ (0.75- 0.78), temperature (20- 35$^{o}$C) and specified spice addition levels were monitored to predict optimum combination for this study. Sensory evaluation for spiced tea was carried out by presenting samples of treated tea extracts to a fifty-two man panelist.

## 3.6 Sample coding and interpretation

All the samples required for this work were coded in line with the originating location for convenience and easy identification (see Table 3.1).

## 3.7 Chemical analyses of tea and coffee

### 3.7.1 Total phenolics

Portion of the infused tea (1 mL) and coffee (1 mL) solution were dispensed into a volumetric flask, 4 mL of distilled water and 5 mL of tartrate solution (1 g of $FeSO_4$

**Table 3.1 Sample codes and interpretation**

| Source | Code | Product | Interpretation |
|---|---|---|---|
| Ibadan | IC**1**B | Tea | Ibadan commercial 1, Black |
| | IC**2**B | Tea | Ibadan commercial 2, Black |
| | IC**3**B | Tea | Ibadan commercial 3, Black |
| | IC**4**B | Tea | Ibadan commercial 4, Black |
| | IC**5**B | Tea | Ibadan commercial 5, Black |
| | IC**6**G | Tea | Ibadan commercial 6, Green |
| | IC**7**G | Tea | Ibadan commercial 7, Green |
| | IC**8**G | Tea | Ibadan commercial 8, Green |
| | IC**9**G | Tea | Ibadan commercial 9, Green |
| | IC**10**G | Tea | Ibadan commercial 10, Green |
| Mambilla | MC**1**B | Tea | Mambilla commercial 1, Black |
| | MC**2**B | Tea | Mambilla commercial 2, Black |
| | MC**3**B | Tea | Mambilla commercial 3, Black |
| | MC**4**B | Tea | Mambilla commercial 4, Black |
| | MC**5**B | Tea | Mambilla commercial 5, Black |
| | MC**6**G | Tea | Mambilla commercial 6, Green |
| | MC**7**G | Tea | Mambilla commercial 7, Green |
| | MC**8**G | Tea | Mambilla commercial 8, Green |
| | MC**9**G | Tea | Mambilla commercial 9, Green |
| | MC**10**G | Tea | Mambilla commercial 10, Green |
| Ibadan | IGCc**1** | Coffee | Ibadan green commercial coffee 1 |
| | IGCc2 | Coffee | Ibadan green commercial coffee 2 |
| | IBCc3 | Coffee | Ibadan black commercial coffee 3 |
| | IBCc4 | Coffee | Ibadan black commercial coffee 4 |
| Mambilla | MGCc1 | Coffee | Mambilla green commercial coffee 1 |
| | MGCc2 | Coffee | Mambilla green commercial coffee 2 |
| | MBCc3 | Coffee | Mambilla black commercial coffee 3 |
| | MBCc4 | Coffee | Mambilla black commercial coffee 4 |

and 5 g of $KNaC_4H_4O_6$ disolved in 1000 mL distilled water) were added. The mixture was diluted with 25 mL of buffer solution (23.377 g of $Na_2HPO_4$ in 1000 mL of distilled water and 9.078 g of $KH_2PO_4$ in 1000 mL of distilled water in the ratio of 85 and 15% v/v), and the absorbance was measured using UV/Vis spectrophotometer (CESIL CE7200, Cesil Instrumental, Cambridge, England) at 540nm. To calculate the level of total phenolics, the following expression was used (Imran *et al*., 2014):

Total polyphenols (g/100g infused tea) = $\frac{0.3914EVo/V1}{W}$ ….. Equation 3.1

Where,

$E$ = the absorbance reading of the spectrophotometer,

$V_o$ = total volume of the tea/coffee solution (250 mL),

$V_1$ = volume used for the measurement (1 mL), and

$W$ = the dry weight of the tea/coffee sample.

### 3.7.2 Total catechins

A known volume (0.1 mL) of tea was taken and dispensed into a test tube containing (0.9 mL) methanol; following the method reported by Imran *et al*. (2014), but with slight modification.

### 3.8.0 Proximate composition of spice varieties (*Curcuma longa, Xylopia aethiopica* and *Piper guineense*)

Duplicate samples of processed spice varieties (*Curcuma longa*, *Xylopia aethiopica* and *Piper guineense*) were pooled and approximately 50 g portion collected as representative, was analysed for proximate composition according to AOAC, (2012).

### 3.8.1 Moisture content of green tea and black tea

Moisture content determination was done in line with the procedure documented by the Official method of Analytical Chemists. Moisture content was calculated and reported in accordance with AOAC (2012).

$$\% \text{ MC} = \frac{W1 - W2}{W1 - Wo} \times 100 \quad \text{…….} \quad \text{Equation 3.2}$$

Where, $W_0$ = the weight of empty crucible

$W_1$ = weight of crucible plus sample

$W_2$ = weight of crucible plus oven dried sample

% MC= percentage moisture content

### 3.8.2 Crude protein determination in *C. longa, X. aethiopica* and *P. guineense* spice

Crude protein in the spice samples was determined by the semi-micro kjedahl technique involving digestion, distillation and titration (AOAC, 2012).

The percentage Nitrogen in the sample was thus calculated using the formula:

$$\% N = \frac{V2 \times N \times Mn \times Vo \times 100}{W \times V1} \quad \text{……..} \quad \text{Equation 3.3}$$

Where,

$V_0$ = Volume of flask containing the digest

$V_1$ = Volume of digest for steam distillation

$V_2$ = Titre value

N = Normality/ Molarity of HCl used

$M_n$ = Atomic mass of Nitrogen

W = Weight of sample digested in milligram

The crude protein was thereafter evaluated by multiplying percentage Nitrogen and a constant factor of 6.25 i.e (% CP = % N x 6.25) …….. Equation 3.4

### 3.8.3 Ether extracts in *C. longa, X. aethiopica* and *P. guineense* spice

Ether extract of the spices was determined in accordance with the procedure reported in AOAC (2012). The percentage fat content was estimated as:

$$\% \text{ Fat} = \frac{W1 - Wo}{W2} \times 100 \quad \text{……} \quad \text{Equation 3.5}$$

Where,

$W_0$ = initial weight of dry Soxhlet flask

$W_1$ = final weight of oven dried flask plus oil/ fat

$W_2$ = initial weight of sample

### 3.9.0 Microbial analyses of tea and coffee

### 3.9.1 Determination of mycobiota of tea and coffee

Duplicate samples of about 1.0 g of tea and coffee were directly inoculated on Potato-dextrose agar. The agar plates were held at room temperature (27 degree celcius) for 72 hours in mermmette incubator. Fungal growth observed on the plates were scrapped, stored in eppendorf bottles and preserved at refridgeration temperature (≤ 4$^{o}$C) pending DNA extraction and further analyses.

### 3.9.2 DNA sequencing for fungi isolates

#### 3.9.2.1 Extraction of DNA using CTAB method

Harvested fungal isolates stored in eppendorf tube was subjected to DNA extraction according to the method reported by Carter-House *et al*. (2020). DNA concentration in the solution was measured on spectrophotometer at 260 nm and 280 nm and the genomic purity were determined.

#### 3.9.2.2 DNA Electrophoresis

The quality and integrity of the DNA, by size fractionation on 1.0 % agarose gels, was determined by adopting the method of Carter-House *et al*. (2020). The DNA separation was done at 80V for 2 hours. The integrity assessment of the deoxyribonucleic acid (DNA) was visualized and photographed on UV light source.

#### 3.9.2.3 PCR analysis using ITS 1 and ITS 4 primers

PCR analysis was run with a universal primer for fungi called 1TS 1 and ITS 4. The PCR profile used followed Carter-House *et al*. (2020) procedure.

#### 3.9.2.4 Purification of PCR products

The amplicon was further purified before the sequencing using 2M Sodium Acetate wash techniques. To about 10 µL of the PCR product, 1 µL 2M NaAct pH 5.2 was added, followed by 20 µL Absolute ethanol and held at -20 $^{o}$C for 1hr. the mixture was spun at

10,000 rpm for 10 minutes, then washed with 70 % ethanol and air dried. The sample was re-suspended in 5 µL sterile distilled water and kept at 4 $^{o}$C for sequencing.

#### 3.9.2.5 PCR for sequencing

The primer used for the reaction was ITS 1 and ITS 4. The PCR mix used includes 0.5 µL of BigDye Terminator Mix. 1 µL of 5X sequencing buffer, 1 µL of M13 forward primer with 6.5 µL Distilled water and 1 µL of the PCR product making a total of 10 µL. The PCR profile for Sequencing is a rapid profile, the initial rapid thermal ramp to 96 $^{o}$C for 1 minute followed by 25 cycles of Rapid thermal ramp to 96 $^{o}$C for 10 seconds Rapid thermal ramp to 50 $^{o}$C for 5 seconds and rapid thermal ramp to 60 $^{o}$C for 4 minutes, then followed by rapid thermal ramp to 4 $^{o}$C and held till needed. The nucleotide sequences, released in form A, C, T, and G, were compared to those present in the national resource for molecular biology information (NCBI) nucleotide data bases by using the Blast-n algorithm.

### 3.10 Sample preparation for HPLC analysis

Dried tea sample (2 g) was weighed and placed in a glass beaker. About 200 mL boiling water was added and mixed thoroughly to extract the tea. This was allowed to stand for about 30 min. 0.5 mL of the extract was collected and 0.5 mL of methanol added to it. The mixture was vortexed for 1 minute and thereafter evaporated to dryness using nitrogen gas. The residue was reconstituted with 0.5 mL mobile phase and stored in an inert glass vial with fitted cap and kept pending injection into HPLC.

#### 3.10.1 Quantitation of mycotoxin fractions in tea and coffee using high performance liquid chromatography (LC-ESI-MS/MS)

The method used in this study was reported by Malachova *et al*. (2014), but with modification. Positive identification, was confirmed when the ionic ratio agrees with the specified standards of within 30% as stated in European Commission Guidelines (2017), and an applied retention time of ± 0.03 min.

#### 3.10.2 Detection of mycotoxin production by mycotoxigenic fungi in tea in the presence of tropical spices

*Aspergillus flavus* and *Fusarium solani* isolated from tea from different locations were cultured in two percent (2%) yeast extract, 15% sucrose (YES) medium for 10 days at

25$^{o}$C in the dark (Aroyeun *et al*. 2011). Cultures of yeast extract sucrose (YES) medium were extracted with chloroform (1:1 v/v) for 45 minutes on a wrist action shaker (Burrell Scientific LLC, Pittsburgh, USA). The chloroform extract was dried over anhydrous sodium tetraoxosulphate (VI) and after evaporation in vacuo, the residue was reconstituted in 500 µL of benzene.

### 3.10.3 Spice powder addition on aflatoxin $B_1$ ($AfB_1$) and deoxynivalenol (DON) toxins

The factors and each levels used in this experiment included $a_w$ (0.75 – 0.78), temperature (20 – 35 $^{o}$C) and level of spice powder added were 1000 µg/g, 2000 µg/g, 3000 µg/g and 4000 µg/g. This design was adapted in Aroyeun's report (Aroyeun *et al.*, 2011). To determine the effect of each environmental factor simultaneously on reduction of Aflatoxin $B_1$ and Deoxynivalenol toxins, hence a 3 factor, 4 level Box-Behnken designs (BBD) was used. The variable levels were coded -1, 0, +1, +2. The coded and actual values used in the BBD are shown in Table 3.2. All experimental test runs were done in duplicate and analysed by multiple linear regression, using general full factorial design (Design Expert software version 12, Minneapolis).

#### 3.10.3.1 Preparation of standard solution of $AfB_1$

Aflatoxin $B_1$ standard was purchased from chemical company (St. Luois, MO, USA). A standard stock solution, 100 µg/mL, was prepared by dissolving 1 mg of Aflatoxin $B_1$ in 100 mL of toluene: acetic acid (99:1). Standard working solution of 1 µg/mL was obtained by dissolving 10 µL of the stock solution in 990 µL of toluene.

### 3.10.4 Addition of powder extract of *Curcuma longa*, *Xylopia aethiopica* and *Piper guineense* on Aflatoxin $B_1$ spiked tea

#### 3.10.4.1 Spiking of the infused tea

Aflatoxin $B_1$-free sample of tea (1 kg) and 10 mL of tea beverage held in a 250 mL Erlenmeyer flask were spiked with standard solution of $AfB_1$ at 5 part per billion (5 ppb). To prepare 5 ppb of the working solution, 5 µL was dissolved in 1 litre of toluene (mobile phase). Spiking was carried out in duplicate and a blank sample of tea and tea beverage containing no $AfB_1$ served as control.

### 3.10.4.2 Spices addition and reduction of $AfB_1$

The effects of *Curcuma longa*, *Xylopia aethiopica* and *Piper guineense* spices on spiked samples of tea/ tea beverage were evaluated using different concentrations of screened and aflatoxin $B_1$ free spice powder- 1000 µg/g, 2000µg/g, 3000 µg/g and 4000 µg/g. The tea was infused in hot water and allowed to equilibrate for 5 hours. Thereafter, quantitation of $AfB_1$ was done using HPLC (Malachova *et al.*, 2014). Reduction efficiency (RE) of the selected spices on $AfB_1$ was calculated as:

$$RE = \frac{Xo - X1}{Xo} \times 100 \quad \text{.....} \quad \text{Equation 3.6}$$

Where, Xo = Quantified contamination level (spiked), $X_1$= quantified reduction result.

**Table 3.2**: **Experimental design for the treatments ($AfB_1$ and DON)**

| Variables | | -1 | 0 | 1 | 2 |
|---|---|---|---|---|---|
| Aw | X1 | 0.75 | 0.76 | 0.77 | 0.78 |
| T°C | X2 | 20 | 25 | 30 | 35 |
| Spice addition (µg/g) | X3 | 1000 | 2000 | 3000 | 4000 |

### 3.10.4.3 Crude powder of *Curcuma longa*, *Xylopia aethiopica* and *Piper guineense* on deoxynivalenol (DON) spiked tea

### 3.10.4.4 Preparation of stock solution of DON (1000 µg/mL)

The stock solution of deoxynivalenol was prepared by adding 1 mL ethyl acetate and methanol (95:5) to 1 mg DON standard product (AOAC, 2012).

### 3.10.4.5 Preparation of working solution of deoxynivalenol

Standard working solution (0.5 ppm, 1 ppm, 2 ppm and 4 ppm) of DON were prepared. 250 µL of 100 µg/mL DON standard was dried down in acetonitrile in a salinized 30 mL amber bottle. Obtained residue was dissolved in 25 mL of mobile phase. This was vortexed for 1 minute. About 750 µL of the mix was transferred into an amber auto sample vial and capped. Standard injection of 250 µL was injected into the HPLC giving a standard of 2 ppm.

### 3.10.4.6 Spiking of infused tea

DON-free sample of tea (1 kg) and a 10 mL of tea beverage held in a 250 mL Erlenmeyer flask were spiked with standard solution of DON at 5 part per billion (5 ppb). To prepare 5 ppb of the working solution, 5 µL was dissolved in 1 litre of acetonitrile (mobile phase). Infused tea were presented in duplicates and contaminated or spiked. Blank tea (control) beverage without contamination served as control.

### 3.10.4.7 Spices addition and reduction of DON

The effect of *C. longa*, *X. aethiopica* and *P. guineense* spices on spiked samples of tea was done using different concentrations of screened and deoxynivalenol free spice powder- 1000 µg/g, 2000 µg/g, 3000 µg/g and 4000 µg/g. The tea was infused in hot water and allowed to equilibrate for 5 hours. Thereafter, quantitation of DON was done using HPLC (Malachova *et al.*, 2014). Reduction efficiency (RE) of the spices on deoxynivalenol toxin was calculated as in above.

## 3.11 Analysing the functional groups of phytoactive compounds present in the selected spices

Dried powder of different spice extracts each of *Curcuma longa*, *Xylopia aethiopica* and *Piper guineense* materials were used for FT-IR analysis. About 10 mg of the milled

powder was encapsulated in 100 g of Potassium bromide (KBr) pellet, an alkali halide, in order to prepare translucent sample discs. The formed pellets of each spice specimen was loaded in FT-IR spectroscope (Schimadu, IR affinity 1, Japan), with a scan range of 400 – 4000/cm and a resolution of 4/cm (Ashokkumar and Ramaswamy, 2014). This was further elucidated by the aid of NMReady, Pro 60N (Luvincia *et al*., 2019).

### 3.12 Sensory analysis of spice tea blend

Approval of the ethical committee at the Institute for Advanced Medical Research and Training (IAMRAT), a body responsible for managing ethical issues, College of Medicine, University of Ibadan, was sought and granted. This approval was conveyed via assigned number: UI/EC/22/0087 (see appendix J). Acceptability was determined by means of a 1 to 9 point standard hedonic rating scale and sensory quality analysis (appearance, colour, smell/aroma, taste and overall acceptability) were evaluated with 1- dislike extremely, 5- neither like nor dislike and 9- like extremely. The samples were judged by a panel of 52-persons comprising 30 male and 22 female who were research fellows and non-research staff of the Cocoa Research Institute of Nigeria, Ibadan. They were regular tea consumers. The panelists were notified about the type of samples. At each session and for each sample, all formulations were presented in separate cups at a temperature of 57± 2$^{o}$C in a well illuminated sensory laboratory. Water was provided for mouth rinsing in between samples. The samples were coded with three random numbers (Iwe, 2013).

### 3.13 Statistical analysis of collected data

Sorensen's index of similarity was used to establish the presence of similar species at different sampling points and was expressed using the formula:

$$S = \frac{2c}{a+b} \times 100$$ (Aroyeun *et al.* 2011) …….. Equation 3.7

Where;

a: the number of species at one site

b: number of species at the other site

c: common species to both location.

Data obtained for each parameter were analysed for variance (ANOVA), using R statistical package version 4.1.3 and SAS, version 9.2 at $p<0.05$ for significance testing. Duncan Multiple range test (DMRT) was used to separate the means.

# CHAPTER FOUR

# RESULTS AND DISCUSSION

## 4.1 Total polyphenol and catechin in tea and coffee from Ibadan and Mambilla

### 4.1.1 Total phenol and catechin in tea

Table 4.1 presents the result of quantitation on amounts of polyphenol and catechin in tea from Mambilla and Ibadan. Minimum level of polyphenol recorded was 2.324 mg/g while the highest value recorded was 13.092 mg/g. In the case of catechin, this ranged from 0.0087- 0.3388 mg/g. Some of the black teas contaminated with Aflatoxin $B_1$ (IC**2**B, MC**2**B) also had high polyphenol content except for MC**5**B, with low polyphenol value and high catechin content. Generally, green tea had polyphenol levels ranging from 8.924- 13.093 mg/g. Reported values for phenolics and catechin in this work slightly differ from those reported by Abourashed *et al*. (2016) who observed a higher phenolic content (14 -21 mg/g) in green teas and (8.42- 17.62 mg/g) in black teas. Catechin content in green tea ranged from 0.0087- 0.02 mg/g. Likewise, polyphenol content in black teas ranged from 2.3243- 7.8757 mg/g and catechin level being 0.0097- 0.0123 mg/g. This shows that phenolic content in green tea was higher than phenolic level of black teas. Very low values of phenol and catechin content were observed in MC**4**G (2.7577/0.0100 mg/g) and MC**5**B (3.0600/0.3388 mg/g).

### 4.1.2 Polyphenol and catechin levels in coffee

Table 4.2 presented the total phenolic and total catechin content of coffee obtained from Mambilla and Ibadan. The total phenolic content ranged between 3.425 and 5.855 mg/g while total catechin ranged between 0.010 and 0.036 mg/g. Significant difference exists between all the coffee samples analysed for total polyphenol and catechin except for total catechin in IBCc3 and IBCc4 which were similar. No significant difference ($p< 0.05$)

**Table 4.1 Total polyphenol and catechin content of green tea and black tea**

| Sample | Polyphenol (mg/g) | Catechin (mg/g) |
|---|---|---|
| IC**1**B | 5.141±0.001$^{q}$ | 0.013±0.0001$^{h}$ |
| *IC**2**B | 7.380±0.001$^{k}$ | 0.012±0.0001$^{hi}$ |
| IC**3**B | 7.568±0.001$^{j}$ | 0.020±0.0000$^{e}$ |
| IC**4**B | 7.876±0.001$^{i}$ | 0.030±0.0000$^{c}$ |
| IC**5**B | 6.886±0.001$^{n}$ | 0.025±0.0000$^{d}$ |
| IC**1**G | 9.610±0.001$^{d}$ | 0.021±0.0010$^{e}$ |
| IC**2**G | 8.924±0.006$^{f}$ | 0.011±0.0015$^{i}$ |
| IC**3**G | 11.051±0.006$^{c}$ | 0.012±0.0000$^{hi}$ |
| IC**4**G | 7.112±0.002$^{l}$ | 0.024±0.0000$^{d}$ |
| IC**5**G | 8.902±0.002$^{g}$ | 0.018±0.0000$^{f}$ |
| MC**1**B | 5.205±0.000$^{p}$ | 0.009±0.0000$^{k}$ |
| *MC**2**B | 7.083±0.006$^{m}$ | 0.010±0.0000$^{j}$ |
| MC**3**B | 5.460±0.001$^{o}$ | 0.009±0.0001$^{k}$ |
| MC**4**B | 2.324±0.001$^{t}$ | 0.010±0.0006$^{j}$ |
| *MC**5**B | 3.061±0.006$^{r}$ | 0.339±0.0001$^{a}$ |
| MC**1**G | 13.092±0.001$^{a}$ | 0.010±0.0000$^{j}$ |
| MC**2**G | 11.922±0.001$^{b}$ | 0.008±0.0001$^{l}$ |
| MC**3**G | 9.130±0.001$^{d}$ | 0.017±0.0000$^{g}$ |
| MC**4**G | 2.758±0.001$^{s}$ | 0.010±0.0000$^{j}$ |
| MC**5**G | 8.765±0.001$^{h}$ | 0.067±0.0000$^{b}$ |

Values are means of triplicate (n= 3) determination. abcd.. superscripts along same column indicates means are significantly different ($p< 0.05$). * - $AfB_1$ contaminated tea

**Table 4.2 Total polyphenol and catechin in green coffee and black coffee**

| Sample | Total Polyphenol (mg/g) | Total Catechin (mg/g) |
|---|---|---|
| IGCc1 | $4.288 \pm 0.006^{e}$ | $0.012 \pm 0.0001^{f}$ |
| IGCc2 | $5.671 \pm 0.001^{b}$ | $0.023 \pm 0.0000^{d}$ |
| IBCc3 | $5.855 \pm 0.006^{a}$ | $0.036 \pm 0.0001^{a}$ |
| IBCc4 | $5.365 \pm 0.001^{c}$ | $0.036 \pm 0.0001^{a}$ |
| MGCc1 | $4.264 \pm 0.001^{f}$ | $0.010 \pm 0.0001^{g}$ |
| MGCc2 | $4.576 \pm 0.001^{d}$ | $0.016 \pm 0.0001^{e}$ |
| MBCc3 | $3.425 \pm 0.001^{h}$ | $0.027 \pm 0.0001^{c}$ |
| MBCc4 | $3.782 \pm 0.001^{g}$ | $0.032 \pm 0.0000^{b}$ |

Values are means of triplicate (n= 3) determination. abcd.. superscripts along same column indicates values are statistically significant ($p < 0.05$).

exists between black coffee obtained from Ibadan; IBCc3, IBCc4 and green coffee, IGCc2 in terms of total polyphenol content. This position was similar with other samples, as results on Table 4.2 indicated. Total catechin content of black coffee from Ibadan differs from ($p< 0.05$) black coffee from Mambilla. Values observed for phenolic content in green coffee slightly agreed with 4.55- 34.43mg/g reported by Perdani *et al*. (2019).

### 4.1.3 Effect of spice blends on total polyphenol content of tea and coffee

Total polyphenol content of black teas blended with varying concentrations of *C. longa, X. aethiopica* and *P. guineense* powder extract is presented on Table 4.3. In comparison with the control (No spice) tea, there was an increase in total polyphenol content (5.48-6.50 mg/g) of the blend as *C. longa* proportion increased from 1000 ppm to 4000 ppm. Similar trend was observed for *X. aethiopica* spice blends as it increased from 6.22- 7.10 mg/g. Reverse was the case with *P. guineense*. As concentration of *Piper guineense* spice increased from 1000 ppm to 4000 ppm, total polyphenol content decreased from 5.05-4.62 mg/g. The total polyphenol content of tea spice blends of *Curcuma longa* and *Xylopia aethiopica* were higher than control showing more antioxidant capacity to reduce fungi metabolite contaminants in tea. Effect of adding spices to green tea and green coffee as shown on Table 4.4 revealed a gain and positivity in this venture. Blending of these spices certainly enhance the phenolic content of tea and coffee, making the obtained product to posses more phenolic materials comparable with reports from Abourashed *et al*. (2016) and Perdani *et al*. (2019). Incorporation of some tropical spices into tea and coffee improved the quality of phenolic matters of tea and coffee obtained from Nigerian markets. Less effectiveness of *piper guineense* spice as shown on Table 4.29 and Table 4.30 can be attributed to low density of phenolic materials in the spice. The trend for coffee spice blends was similar to results obtained with tea spice blends at the same spice concentrations.

## 4.2 Effect of spice blends on the proximate composition of tea and coffee

### 4.2.1 Moisture content

The result of proximate composition of tea (*Camellia sinensis*) is presented on Table 4.5. The mean moisture content of all the tea samples ranged from 5.46% - 7.51% with

retailed black tea (MC**5**B) having the highest moisture (7.51%) while sample MC**6**G-green tea, have the lowest moisture content (5.46%). The moisture content of black tea from Mambilla, MC**4**B, and black tea, from Ibadan, IC2B, was 6.87% and 7.00% respectively. Likewise, the mean moisture content of green tea commodity from mambilla (MC**6**G) and Ibadan, (IC**6**G), were 5.46% and 5.51%. This result agreed with the report by Muhammed *et al* (2013), who reported very high percentage moisture content in black tea in comparison with green tea. Processing and location may be responsible for the variation in moisture content. High moisture content aids microbial activities, oxidation-reduction processes and fungal growth. The highest percentage of moisture were observed in black tea samples as compared to green tea commodities. Significant difference ($p < 0.05$) exists in the moisture content of green teas and black teas. The moisture content of black tea-spice blends on Table 4.7 was low. All the blends have moisture content lees than 6.87 % and this agreed with previous results as discussed above.

Table 4.7 showed the moisture content of spice-tea blends. *Curcuma longa* spice blended into tea commodity at 4000 ppm had lower moisture content (5.85%) compared with tea commodity without spice blends (6.87%). The same trend was reported for *C. longa* spice blended in to tea at 3000 ppm. This therefore indicated a tendency of *Curcuma* spice in reducing moisture content and thus mitigates microbial activity in the commodity. *Xylopia aethiopica* spice, at 3000 ppm, incorporated into tea commodity also reduced moisture level of the spiced tea. Generally, all the produced spiced tea had low moisture content (< 7.0%).

Similarly, Table 4.8 reveals the impact of *Curcuma longa*, *Xylopia aethiopica* and *Piper guineense* spices on coffee commodity. *Curcuma longa* and *Piper guineense* spices (4000 ppm) reduced moisture content of coffee to 5.81% and 5.80% respectively. This same trend was observed with spice concentration incorporated at 3000 ppm for *Curcuma longa* and *Piper guineense*. Also, 1000 ppm *C. longa* and *Piper guineense* reduced moisture content to 6.02% and 6.00% respectively. This affirmed the tendency of reducing exposure of coffee to microbial attack. Meanwhile, *Xylopia aethiopica* spice increased moisture content of coffee commodity across all blends.

**Table 4.3: Total polyphenolic content of spiced black tea and spiced black coffee blends**

| Sample | Black Tea Tp (mg/g) | Black Coffee Tp (mg/g) |
|---|---|---|
| No spice | 5.21[b] | 3.43[b] |
| 1000Cl | 5.48[c] | 3.53[b] |
| 1000Xa | 6.22[d] | 3.82[c] |
| 1000Pg | 5.05[b] | 3.22[b] |
| 3000Cl | 5.97[c] | 3.78[c] |
| 3000Xa | 6.55[d] | 4.04[d] |
| 3000Pg | 4.89[a] | 3.1[a] |
| 4000Cl | 6.5[d] | 4.08[d] |
| 4000Xa | 7.1[e] | 4.44[e] |
| 4000Pg | 4.62[a] | 3.03[a] |

Values are means of triplicate determination. [abcd] superscripts along same column indicates values are significantly different ($p < 0.05$). Cl-*Curcuma longa*, Xa- *Xylopia aethiopica*, Pg- *Piper guineense*, Tp- Total polyphenol

**Table 4.4: Total polyphenolic content of spiced green tea and spiced green coffee blends**

| Sample | Green Tea Tp (mg/g) | Green Coffee Tp (mg/g) |
|---|---|---|
| No spice | 17.08[b] | 19.97[b] |
| 1000Cl | 17.45[b] | 20.17[c] |
| 1000Xa | 19.09[d] | 19.46[b] |
| 1000Pg | 15.92[a] | 18.76[a] |
| 3000Cl | 18.84[c] | 21.32[d] |
| 3000Xa | 19.42[e] | 20.58[c] |
| 3000Pg | 15.86[a] | 18.64[a] |
| 4000Cl | 19.37[e] | 21.62[d] |
| 4000Xa | 19.97[e] | 21.98[d] |
| 4000Pg | 15.49[a] | 18.57[a] |

Values are means of triplicate determination. [abcd] superscripts along same column indicates values are significantly different ($p < 0.05$). Cl-*Curcuma longa*, Xa- *Xylopia aethiopica*, Pg- *Piper guineense*, Tp- Total polyphenol

**Table 4.5: Proximate composition of tea**

| Sample | Moisture (%) | Protein (%) | Fat (%) | Fibre (%) | Ash (%) | CHO (%) |
|---|---|---|---|---|---|---|
| IC**6**G | 5.51±0.00$^{f}$ | 1.48±0.05$^{g}$ | 1.83±0.04$^{a}$ | 16.89±0.01$^{b}$ | 4.23±0.03$^{f}$ | 70.06±0.09$^{e}$ |
| IC**7**G | 5.77±0.03$^{e}$ | 1.52±0.04$^{f}$ | 1.66±0.03$^{h}$ | 16.71±0.00$^{c}$ | 4.33±0.03$^{e}$ | 70.01±0.30$^{f}$ |
| MC**6**G | 5.46±0.05$^{g}$ | 1.42±0.03$^{h}$ | 1.73±0.03$^{g}$ | 17.18±0.01$^{a}$ | 4.14±0.04$^{g}$ | 70.07±0.10$^{d}$ |
| MC**7**G | 5.31±0.08$^{h}$ | 1.55±0.08$^{e}$ | 1.91±0.06$^{e}$ | 16.55±0.05$^{d}$ | 4.34±0.02$^{e}$ | 70.34±0.00$^{b}$ |
| IC**1**B | 6.67±0.00$^{d}$ | 1.82±0.00$^{a}$ | 3.11±0.00$^{d}$ | 13.55±0.05$^{f}$ | 4.38±0.00$^{d}$ | 70.47±0.05$^{a}$ |
| IC**2**B | 7.00±0.01$^{b}$ | 1.68±0.01$^{d}$ | 3.24±0.01$^{c}$ | 13.22±0.00$^{h}$ | 4.56±0.01$^{c}$ | 70.30±0.01$^{c}$ |
| MC**4**B | 6.87±0.00$^{c}$ | 1.74±0.00$^{c}$ | 3.45±0.05$^{a}$ | 13.47±0.02$^{g}$ | 4.62±0.00$^{b}$ | 69.85±0.05$^{g}$ |
| MC**5**B | 7.51±0.01$^{a}$ | 1.78±0.005$^{b}$ | 3.34±0.04$^{b}$ | 13.48±0.01$^{e}$ | 5.43±0.03$^{a}$ | 68.46±0.01$^{h}$ |

Values are means of triplicate determination, abc... values in the same column bearing different superscripts are significantly different ($p< 0.05$).

### 4.2.2 Crude protein

In Table 4.5, protein content of the processed tea ranged from 1.42% - 1.82%. Lowest mean value for protein was recorded in green tea, MC**6**G, (1.42%), and highest protein value in commodity MC**5**B, (1.77%). These results were in line with report of FatSecret 2022, which presented 1-2% protein content for better quality of commercial tea. Significant difference ($p<0.05$) exists in protein for all tea samples analysed.

## 4.3 Proximate composition of *curcuma longa, xylopia aethiopica* and *piper guineense* powder extract

### 4.3.1 Moisture content

The moisture content of spice powder extract is presented on Table 4.6. Lowest value was reported in *Piper guineense* powder and highest value in *Xylopia aethipica*. There exists a significant difference ($p < 0.05$) between the mean values of the moisture content of the spices. Values of percentage moisture content ranged from 5.39% - 7.25% as XAS>CLS>PGS. Result obtained agreed with the position of Okibe *et al.* (2020), who declared percentage moisture content of 7.35% for *Xylopia aethiopica* powder extract. Also, mean value of percentage moisture for *Piper guineense* as reported by Okeke *et al*. (2018) was 11.13% contrary to result (5.39%) in Table 4.6.

### 4.3.2 Crude protein

The protein content reported on Table 4.6 was in the order PGS>XAS>CLS. Mean values of the percentage protein content ranged from 7.79% -17.79%. Significant difference ($p< 0.05$) exists between *Curcuma longa* powder extract and the other spices. Taoheed *et al*. (2017), reported 1.83% for *Curcuma*, Okibe *et al*. (2020), 9.37% for *Xylopia* and Okeke *et al*. (2018), reported 12.62% for *Piper guineense*.

### 4.3.3 Ash

Table 4.6 presents the percentage ash content of the spices. Mean values for ash ranged from 3.12% to 6.11% in the order XAS>PGS>CLS. Significant difference ($p < 0.05$) exists between XAS and CLS, while PGS was similar to the other two spices. Taoheed *et*

**Table 4.6: Proximate composition of *C. longa*, *X. aethiopica* and *P. guineense* spices**

| Sample | Moisture (%) | Protein (%) | Fat (%) | Fibre (%) | Ash (%) | CHO (%) |
|---|---|---|---|---|---|---|
| XAS | 7.25±0.10$^{a}$ | 15.52±0.10$^{b}$ | 3.82±0.12$^{c}$ | 5.54±0.10$^{a}$ | 6.61±0.10$^{a}$ | 61.26±0.07$^{b}$ |
| CLS | 5.57±0.06$^{b}$ | 7.79±0.01$^{c}$ | 6.88±0.07$^{b}$ | 2.03±0.03$^{c}$ | 3.12±0.04$^{c}$ | 74.61±0.10$^{a}$ |
| PGS | 5.39±0.10$^{c}$ | 17.79±0.20$^{a}$ | 7.71±0.05$^{a}$ | 3.53±0.10$^{b}$ | 4.62±0.10$^{b}$ | 60.96±0.05$^{c}$ |

Values are means of triplicate (n= 3) determination, mean in the same column bearing different superscripts are statistically significant ($p< 0.05$). XAS- *Xylopia aethiopica* spice, CLS- *Curcuma longa* spice, PGS- *Piper guinense* spice.

**Table 4.7: Proximate composition of spiced-black tea**

| Sample | % Mositure | % Protein | % Fat | % Fibre | % Ash | % CHO |
|---|---|---|---|---|---|---|
| MC**4**B | 6.87±0.04$^{h}$ | 1.74±0.00$^{i}$ | 3.45±0.00$^{e}$ | 13.47±0.02$^{a}$ | 4.62±0.04$^{d}$ | 69.85±0.00$^{c}$ |
| MC**4**B.1000Cl | 6.20±0.05$^{c}$ | 2.05±0.00$^{h}$ | 3.77±0.00$^{c}$ | 13.38±0.02$^{b}$ | 4.44±0.00$^{f}$ | 70.16±0.05$^{a}$ |
| MC**4**B.1000Xa | 6.85±0.04$^{h}$ | 2.50±0.00$^{f}$ | 3.35±0.03$^{g}$ | 13.21±0.00$^{e}$ | 4.68±0.01$^{c}$ | 69.41±0.08$^{d}$ |
| MC**4**B.1000Pg | 6.72±0.01$^{d}$ | 2.88±0.00$^{d}$ | 3.52±0.05$^{d}$ | 13.08±0.00$^{d}$ | 4.58±0.00$^{d}$ | 69.22±0.06$^{e}$ |
| MC**4**B.3000Cl | 6.10±0.04$^{b}$ | 2.40±0.08$^{g}$ | 4.00±0.04$^{b}$ | 13.22±0.00$^{e}$ | 4.21±0.05$^{g}$ | 70.07±0.03$^{b}$ |
| MC**4**B.3000Xa | 6.80±0.00$^{g}$ | 2.61±0.00$^{e}$ | 3.40±0.00$^{f}$ | 13.28±0.02$^{d}$ | 4.72±0.03$^{b}$ | 69.19±0.03$^{e}$ |
| MC**4**B.3000Pg | 6.75±0.02$^{e}$ | 2.95±0.00$^{c}$ | 3.77±0.00$^{c}$ | 13.15±0.00$^{f}$ | 4.50±0.00$^{e}$ | 68.88±0.00$^{f}$ |
| MC**4**B.4000Cl | 5.85±0.00$^{a}$ | 2.55±0.05$^{f}$ | 4.35±0.05$^{a}$ | 13.01±0.00$^{g}$ | 4.18±0.00$^{g}$ | 70.06±0.02$^{b}$ |
| MC**4**B.4000Xa | 6.75±0.03$^{e}$ | 3.05±0.03$^{b}$ | 3.50±0.03$^{d}$ | 13.33±0.00$^{c}$ | 4.80±0.06$^{a}$ | 68.57±0.00$^{g}$ |
| MC**4**B.4000Pg | 6.79±0.00$^{f}$ | 3.20±0.00$^{a}$ | 4.00±0.04$^{b}$ | 13.23±0.02$^{e}$ | 4.45±0.03$^{f}$ | 68.33±0.03$^{h}$ |

Values are means of triplicate determination, abc... values in the same column bearing different superscripts are significantly different ($p< 0.05$).

**Table 4.8 Proximate composition of black coffee spice blends**

| Sample | Moisture % | Protein % | Fat % | Fibre % | Ash % | CHO % |
|---|---|---|---|---|---|---|
| MBCc3 | 6.05±0.00$^{f}$ | 11.67±0.00$^{g}$ | 3.29±0.01$^{j}$ | 4.68±0.03$^{d}$ | 3.49±0.01$^{g}$ | 70.82±0.04$^{d}$ |
| MBCc3.1000Cl | 6.02±0.00$^{e}$ | 11.32±0.01$^{h}$ | 3.44±0.02$^{f}$ | 4.53±0.02$^{f}$ | 3.41±0.04$^{h}$ | 71.28±0.05$^{c}$ |
| MBCc3.1000Xa | 6.10±0.01$^{g}$ | 11.73±0.01$^{f}$ | 3.30±0.03$^{i}$ | 4.73±0.01$^{c}$ | 3.52±0.00$^{f}$ | 70.61±0.00$^{f}$ |
| MBCc3.1000Pg | 6.00±0.01$^{d}$ | 11.77±0.01$^{e}$ | 3.46±0.02$^{e}$ | 4.61±0.00$^{e}$ | 3.53±0.00$^{e}$ | 70.63±0.00$^{e}$ |
| MBCc3.3000Cl | 5.96±0.02$^{c}$ | 11.22±0.02$^{i}$ | 3.56±0.02$^{d}$ | 4.33±0.03$^{i}$ | 3.34±0.04$^{i}$ | 71.59±0.03$^{b}$ |
| MBCc3.3000Xa | 6.18±0.02$^{h}$ | 11.81±0.02$^{d}$ | 3.35±0.02$^{h}$ | 4.75±0.00$^{b}$ | 3.61±0.03$^{d}$ | 70.30±0.03$^{g}$ |
| MBCc3.3000Pg | 5.93±0.01$^{b}$ | 11.90±0.03$^{c}$ | 3.81±0.03$^{c}$ | 4.50±0.00$^{g}$ | 3.74±0.03$^{c}$ | 70.12±0.04$^{h}$ |
| MBCc3.4000Cl | 5.81±0.02$^{a}$ | 11.06±0.01$^{j}$ | 4.00±0.04$^{b}$ | 4.10±0.03$^{j}$ | 3.21±0.00$^{j}$ | 71.82±0.05$^{a}$ |
| MBCc3.4000Xa | 6.30±0.03$^{i}$ | 12.10±0.03$^{b}$ | 3.40±0.01$^{g}$ | 4.88±0.05$^{a}$ | 3.98±0.00$^{a}$ | 69.34±0.01$^{j}$ |
| MBCc3.4000Pg | 5.80±0.02$^{a}$ | 12.20±0.03$^{a}$ | 4.23±0.03$^{a}$ | 4.45±0.02$^{h}$ | 3.95±0.00$^{b}$ | 69.37±0.02$^{i}$ |

Values are means of triplicate determination, abc... values in the same column bearing different superscripts are significantly different ($p< 0.05$).

*al*. (2017) reported 3.04% for CLS, Okibe *et al*. (2020), reported 3.70% for XAS and Okeke *et al*. (2018), reported 4.51% for PGS. Close similarities exists in the obtained results when compared with the reports from other researchers.

### 4.3.4 Crude fiber

Results of crude fiber as presented in Table 4.6 showed that significant difference ($p< 0.05$) exists in the three spices. Lowest mean value observed for fiber is 2.03% and highest mean value stands at 5.54%. Result (1.95%) reported by Taoheed *et al.*, (2017) for CLS was similar to value on Table 4.6.

### 4.3.5 Crude fat

Percentage crude fat for the spices used in this study is presented in Table 4.6. The mean value for fat content is in the order XAS<CLS<PGS. There is significant difference ($p <0.05$) in fat content of all the spices. Okeke *et al.*, (2018) reported 7.60%, a result close to 7.71% shown in Table 4.6.

## 4.4 Effects of storage conditions on mycobiota of tea and coffee

### 4.4.1 Mycobiota of tea and coffee under different storage conditions

Mycobiota of screened tea and coffee stored at different conditions was presented in Table 4.9. Growth of micro-organisms, especially toxigenic fungi, and other moulds was observed. Generally, fungi genera such as *Aspergillus* spp., *Fusarium* spp., *Gliocladium* spp, *Penicillium* spp and *Rhizopus* spp were found growing on tea and coffee stored at temperature of 27± 2 $^{o}$C and relative humidity of 75± 5 %. Tea and coffee at the storage condition of 15± 5 $^{o}$C and relative humidity of 45± 5 % had no visible growth of fungi during the period of storage. This is an indication that fungi growth is aided by suitable temperature and optimum relative humidity. This result was in agreement with earlier studies (ICCO, 1993), on major contaminating micro organisms of tea and coffee. The identified organisms, *Aspergillus* and *Penicillium*, are majorly storage fungi (Table 4.9). Dehumidified atmosphere storage conditions; temperature (15± 5 $^{o}$C) and relative humidity, RH, (45± 5 %), employed hindered growth of mycotoxigenic fungi. This

method is expensive and impracticable for handlers of tea and coffee commodities especially in the developing countries (Hernandez and Martinez, 2018).

### 4.4.2 Nucleotide sequence for fungi isolate from tea and coffee

Figure 4.1 presents the nucleotide sequence obtained from *Aspergillus* spp and its variations, implying a difference in *Aspergillus* strains. The *Aspergillus* from Ibadan and Mambilla fall under the same clade, this could be linked to genotypical similarity of the strains. Variant strains of Aspergillus spp such as *Aspergillus flavus* strain RCBBR_AEANW2 and *Aspergillus flavus* strain ND28, were identified. Figure 4.2 presents various strains of *Aspergillus niger*, namely, *Aspergillus niger* WIKO- NG, *Aspergillus niger* T-A7 and *Aspergillus niger* RMUAN75. The deletion in nucleotide from the alignments is the difference in each genotype (Oyelakin *et al*., 2016). This result suggests possibility of new shades of toxin metabolites resulting from newer strains of fungi in tea and coffee.

**Table 4.9 Six months storage for tea and coffee at room temperature**

| Week | Mould growth (Molecular analysis) | Group/No of identified organism |
|---|---|---|
| 1 | Nil | |
| 2 | Nil | |
| 3 | Nil | |
| 4 | *A. flavus, A. niger, A foetidus, Fusarium. spp* | 4 |
| 5 | *A. flavus, Fusarium spp., A. welwitchiae* | 3 |
| 6 | *Penicillium spp., A. turbingensis, A. niger* | 3 |
| 7 | *Gliocladium cibotii, Rhizopus delemar* | 2 |
| 8 | *Aspergillus niger, A. foetidus, A. turbingensis* | 3 |
| 9 | *Fusarium spp., Pichia kudriavzevii, G. cibotii* | 3 |
| 10 | *Rhizopus delemar, S. racesmosum, A. niger* | 3 |
| 11 | *Penicillum spp., A. flavus, A. niger* | 3 |
| 12 | *A. welwitschiae, A. foetidus, A. niger* | 3 |
| 13 | *Fusarium spp., Penicillum spp.* | 2 |
| 14 | *A. turbingensis, A. niger, A. flavus* | 3 |
| 15 | *A.flavus, A. foetidus, A. niger, Fusarium sp* | 4 |
| 16 | *Aspergillus foetidus, A. turbingensis, A. niger* | 3 |
| 17 | *Aspergillus flavus, A. niger, A. welwitschiae* | 3 |
| 18 | *Asperillus flavus, A. niger, A. welwitschiae* | 3 |
| 19 | *Aspergillus niger, A. turbingensis, A. flavus* | 3 |
| 20 | *Aspergillus welwitschiae, A. turbingensis* | 2 |
| 21 | *Aspergillus flavus, Penicillum spp* | 2 |
| 22 | *Aspergillus spp., Fusarium spp., Penicillum sp* | 3 |
| 23 | *Penicillum spp., Fusarium spp., Asprgillus spp* | 3 |
| 24 | *Penicillum spp., Fusarium spp., Aspergillus sp.* | 3 |

**Figure 4.1 Nucleotide sequence of fungi Isolate from tea**

36.

CCCGTTATTTCCTTCCATGCATCCGACGTGTCAAGCTGTGGAAACGATTGATTT
GCGTTCGGCAAGCGCCGGCCGGGCCTACAGAGCGGGTGACAAAGCCCCATAC
GCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTGGGGCCCGTCCCCCCCGG
AGAGGGGACGACGACCCAACACACAAGCCGTGCTTGATGGGCAGCAATGACG
CTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAG
ACTCGATGATTCACGGAATTCTGCAATTCACACTAGTTATCGCATTTCGCTGC
GTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGAT
TGCGATACAATCAACTCAGACTTCACTAGATCAGACAGAGTTCGTGGTGTCTC
CGGCGGGCGCGGGCCCGGGGCTGAGAGCCCCCGGCGGCCATGAATGGCGGGC
CCGCCGAAGCAACTAAGGTACAGTAAACACGGGTGGGAGGTTGGGCTCGCTA
GGAACCCTACACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTG
TTACGACTTTTTACTTCCA

Aspergillus flavus strain RCBBR_AEANW2

37.

CGGGTGACAAAGCCCCATACGCTCGAGGATCGGACGCGGTGCCGCCGCTGCC
T
TGGGGCCCGTCCCCCCCGGAGAGGGGACGACGACCCAACACACAAGCCGTGC
T
TGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGC
G
CAATGTGCGTTCAAAGACTCGATGATTCACGGAATTCTGCAATTCACACTAGT
TAT
CGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAA
AGT
TTTAACTGATTGCGATACAATCAACTCAGACTTCACTAGATCAGACAGAGTTC
GTG
GTGTCTCCGGCGGGCGCGGGCCCGGGGCTGAGAGCCCCCGGCGGCCATGAAT
GG CGGGCCCGCCGAAGCAACTAAG

Aspergillus flavus strain ND28

39.

TGGAAAAAATTTGNAAAANNTCNNTANGCGCCGGNCANATGTGACAAATCCC
CATACGCTCGAGGATNNNTTTTTNTGCCGCCNCTGCCTTTCGGGCCCGTCCCC
CCGGANAGGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAA
TGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTT
CAAAGACTCGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTC
GCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAA
CTGATTGCATTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTG
GGGTCTCCGGCGGGCACGGGCCCGGGGGGCAAAGGCGCCCCCCCGGCGGCCG
ACAAGCGGCGGGCCCGCCGAAGCAACAGGGTATAATAGACACGGATGGGAG
GTTGGGCCCAAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTAC
GGAAACCTTGTTACGACTTTTACTTCCA

Aspergillus niger strain HM81 s MT609916.1

28.

TCCTTGGTTCCTTCCATGAATCCGAGGTCAACCTGGGAAAAATGGTTGGAAAA
CGTCGGCAGGCGCCGGCCAATCCTACAGAGCATGTGACAAAGCCCCATACGC
TCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGAGA
GGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTC
GGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACT
CGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCGTTC
TTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCA
TTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTCTCCG
GCGGGCACGGGCCCGGGGGGCAAAGGCGCCCCCCCGGCGGCCGACAAGCGG
CGGGCCCGCCGAAGCAACAGGGTATAATAGACACGGATGGGAGGTTGGGCCC
AAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTT
GTTACGACTTTTTACTTCCN

Aspergillus sp. voucher jxbr-03

39*.

CCTTCCATGAATCCGAGGTCAACCTGGGAAAAATGGTTGGAAAACGTCGGCA
GGCGCCGGCCAATCCTACAGAGCATGTGACAAAGCCCCATACGCTCGAGGAT
CGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGAGAGGGGGAC
GGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTCGGACAGG
CATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGAT
TCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCGTTCTTCATCG
ATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCATTCAATC
AACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTCTCCGGCGGGCA
CGGGCCCGGGGGGCAAAGGCGCCCCCCCGGCGGCCGACAAGCGGCGGGCCC
Aspergillus sp. isolate AUMS56

33.

TACGCTCGAGGACGCTGCCTTTCGGGCCCGTCCCCCCGGAGAGGGGGACGGCGACC

CAACACACAAGCCGGGCTNATGANGCTCGGACAGGCATGCCCCCCGGAATACCNG

GGGGNGCAATGNGCGTTCAAAGACTCGATGATTCANTGAATTCTGNAANNNNNATT

AGTTATCNCATTTCNCTGCGTTCTTCATCNATGCCGGANCCNAGAGATCCNTNGNTG

AANGNTNTANCTGATTGNNTTCAATCNACTCANACTGNNNNCTTTCAGACAGTGTTC

NNGTTGGGGNCTNCGGGGGGCAANNGCGCCCCCNNNGNNGCCGACAAGCGGNGG

GCCCGCCGANGGNATAATAGACACGGATGGGANGNNGGGNCNNAAGGACCCGCA

CTCNGNNATGATCCNTCGNTCANCNNNNNAAANNTNNNTNNNANTTTTNNNTCNNNN

Aspergillus sp. NCIM 947 KR261381.1

**Figure 4.2 Nucleotide sequence of fungi Isolate from coffee**

20.

CCCNTTGTTTCCACCTTGACCTTCAGGATCATTAGATTTGAAAGTTGCTGGATT
ATACTCTTGTACTTTACTTCCTGGGCGAACCAAAGAAAAAGATCCTGAGACCA
GCGTAATATTCCTGCCTAGCAAGCCAGACAGAAAATCACACACATTTTAGGTG
CTCACTGTAATAAAACAGCGATGCGACCCATTACCACATAAACAAATGTTATG
TGTGGGTTTGTGATGATACTGAAGCAGGCGTACTCTATAGAAAAACCATAGA
GTGCAAGCTGCGTTCAAAGACTCGATGATTCACTGAATATGCAATTCACACTA
GTTATCGCACTTTGCTACGTTCTTCATCGATGCGAGAACCAAGAGATCCATTG
TTAAAAGTTGTTTTTTATTAAACTTTATTACTGAATTTCTAGGTTTATTATGAA
GGGTGCTCCTGAAACCAGGAGTGGCATCGATCAAACCCCAGATAGGTCTACC
CATGACCAGTCTGAGTCTCTCAGCCAAATTTTCACAGTGTAGAAGCAATCACT
TACCCCAGAGGAAACCCTAAGAGGTAAGGCGCTTTAACATAATTAATGATCC
TTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTACTTCCA

Rhizopus delemar UICC 524
Rhizopus delemar UICC 121

21.

TTAATTTCCTAACCATGATCCGAGGTCAACCTGGAAAGAATGGTTGGAAAAC
GTCGGCANGCGCCGGCCAATCCTACAGAGCATGTGACAAAGCCCCATACGCT
CGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGAGA
GGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTC
GGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACT
CGATGATTCACTGAATTCTGAATTCACATTAGTTATCGCATTTCGCTGCGTTCT
TCATCGATGCCGGAACCAAGAGACCATTGTTGAAAGTTTTAACTGATTGCATT
CAATCAACTCAGACTGCACGCTTTCAGAGTGTTCGTGTTGGGGTCTCCGGCGG
GCACGGGCCCGGGGGGCAGAGGCGCCCCCCCGGCGGCCGACAAGCGGCGGG
CCCGCCGAAGCAACAGGGTACAATAGACACGGATGGGAGGTTGGGCCCAAA
GGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTAC

Aspergillus niger isolate WIKO-NG MW680955.1

22.
CATGTGACAAAGCCCCATACGCTCGAGGATCGGACGCGGTGCCGCCGCTGCC
TTTCGGGCCCGTCCCCCCGGAGAGGGGGACGGCGACCCAACACACAAGCCGG
GCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGG
GGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCAATTCACAT
TAGTTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCAT
TGTTGAAAGTTTTAACTGATTGCATTCAATCAACTCAGACTGCACGCTTTCAG
ACAGTGTTCGTGTTGGGGTCTCCGGCGGGCACGGGCCCGGGGGGCAGAGGCG
CCCCCCCGGCGGCCGACAAGCGGCGGGCCCGCCGAAGCA

Aspergillus foetidus isolate BM13 MK91

23.
GAAAAATGGTTGGAAAACGTCGGCAGGCGCCGGCCAATCNNTTNAGCATGTG
ACAAAGCCCCATACGCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGG
GCCCGTCCCCCCGGAGAGGGGGACGGCGACCCAACACACAAGCCGGGCTTGA
GGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCA
ATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCAATTCACATTAGTTA
TCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGA
AAGTTTTAACTGATTGCATTCAATCAACTCAGACTGCACGCTTTCAGACAGTG
TTCGTGTTGGGGTCTCCGGCGGGCACGGGCCCGGGGGGCAAAGGCGCCCCCC
CGGCGGCCGACAAGCGGCGGGCCCGCCGAAGCAACAGGGTATAATAGACAC
Fungal sp. isolate Aspergillus niger strain T-A7

24.
AAAANNGGTTGGAAAACGTCGGCAGGCGCCGGCCAATNNNTTNAGNATGTGA
CAAAGCCCCATACGCTCGAGGATCGNANNTNNTGCCGCCGCTGCCTTTCGGG
CCCGTCCCCCCGGAGAGGGGGACGGCGACCCAACACACAAGCCGGGCTTGAG
GGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAA
TGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCAATTCACATTAGTTAT
CGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAA
AGTTTTAA
Aspergillus welwitschiae isolate 1017

25.

CCCNTTGTTTCTTCCTGCATCCGAGGTACAAGCCTGTGGGAAGAATGGTTGGA
AAATGTCGGCNNGCGCCGGCCAATCCTACAGAGCATGTGACAAAGCCCCATA
CGCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGG
AGAGGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGAC
GCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAA
GACTCGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGC
GTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGAT
TGCATTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTC
TCCGGCGGGCACGGGCCCGGGGGGCAGAGGCGCCCCCCCGGCGGCCGACAA
GCGGCGGGCCCGCCGAAGCAACAGGGTACAATAGACACGGATGGGAGGTTG
GGCCCAAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGA
AACCTTGTTACGACTTTTACTTCAA

Aspergillus niger isolate 8, MT588793.1

26.

TTAATTTCCTAACCATGATCCGAGGTCAACCTGGAAAGAATGGTTGGAAAAC
GTCGGCANGCGCCGGCCAATCCTACAGAGCATGTGACAAAGCCCCATACGCT
CGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGAGA
GGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTC
GGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACT
CGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCGTTC
TTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCA
TTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTCTCCG
GCGGGCACGGGCCCGGGGGGCAGAGGCGCCCCCCCGGCGGCCGACAAGCGG
CGGGCCCGCCGAAGCAACAGGGTACAATAGACACGGATGGGAGGTTGGGCCC
AAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTAC

Aspergillus niger isolate RMUAN75 MT550026.1

under the same clade, this could be linked to genotypical similarity of the strains. Variant strains of Aspergillus spp such as *Aspergillus flavus* strain RCBBR_AEANW2 and *Aspergillus flavus* strain ND28, were identified. Figure 4.2 presents various strains of *Aspergillus niger*, namely, *Aspergillus niger* WIKO- NG, *Aspergillus niger* T-A7 and *Aspergillus niger* RMUAN75. The deletion in nucleotide from the alignments is the difference in each genotype (Oyelakin *et al*., 2016). This result suggests possibility of new shades of toxins matabolites resulting from newer strains of fungi in tea and coffee.

### 4.5.0 Mycobiota of tea from Ibadan and Mambilla

Table 4.10 and 4.11 revealed groups of fungi especially *Aspergillus spp*, and *Gliocladium spp*, isolated and identified in tea from Ibadan (Table 4.10). The mixture of these toxigenic fungi and mould did not significantly differ in terms of presence except for *Gliocladium cibotti*. Table 4.11 revealed the dominating presence of *Aspergillus* species in tea obtained from Mambilla. These results agreed with the position of Haas *et al*. (2013) and Hassane *et al*. (2017) on fungal contamination and mycotoxin production in food as a threat to safety and health of consumers. Table 4.12 established the presence of fungi growth in stored green and black tea. *Aspergillus flavus* in green tea was only 10 % but with black tea was as much as 70 %. This partly explains the reason to suspect the presence of aflatoxin contamination, in higher quantity, in black tea. *Fusarium* species was about 30 % population in green tea and 40 % in black tea. The presence of *Fusarium solani* and its consequent increase provided grounds to also suspect deoxynivalenol contamination in black tea. This fungus had been indicted for considerable losses to tea industry (Bhattacharyya *et al.*, 2016). *Aspergillus niger* also grew and proliferate to 30 % in green tea but 40 % in black tea. Note-worthy was the population of *Pichia kudriavzevii* (40 %) in green tea and 20 % in black tea. *Aspergillus welwitschiae* and *Aspergillus foetidus* were present (10 %) both in green tea and black tea.

Table 4.13 showed that relationship exists in terms of fungi found in green and black teas from Mambilla (66.7%). This result is not unconnected to location in terms of climatic influence (Table 4.13) which supported cross contamination of product. Green tea, from Mambilla and Ibadan, as well as Mambilla green and Ibadan black teas, exhibited weak

**Table 4.10 Mycotoxigenic fungi detected in tea from Ibadan, Oyo state, Nigeria**

| Tea | Fungi species detected |
|---|---|
| IC**2**B | *Aspergillus niger*, *Aspergillus foetidus*, *Aspergillus flavus* |
| IC**1**B | *Gliocladium cibotii*, *Aspergillus tubingensis* |
| IC**4**G | *A. niger (isolate WIKO- NG), A. foetidus (Isolate BM 13)* |

**Table 4.11 Mycotoxigenic fungi detected in tea from Mambilla, Taraba state, Nigeria**

| Tea | Fungi specie detected |
|---|---|
| MC**2**B | *Aspergillus niger*, *Aspergillus specie* NCIM 947, *Aspergillus niger* strain HM81s |
| MC**3**B | *Aspergillus niger*, *Aspergillus welwitschiae*, *Aspergillus flavus* |
| MC**1**B | *Aspergillus flavus* |
| MC**5**B | *Aspergillus specie* voucher jxbr-03, *Aspergillus specie* isolate AUMS 56, *Fusarium solani* |
| MC**8**G | *Aspergillus flavus* (isolate RCBBR-AEANW2) |

**Table 4.12 Percentage occurrences of fungi and mould in tea**

| Fungi | Green Tea (%) | Black Tea (%) |
|---|---|---|
| *Aspergillus niger* | 30 | 40 |
| *Aspergillus flavus* | 10 | 70 |
| *Aspergillus welwitschiae* | 10 | 10 |
| *Aspergillus foetidus* | 10 | 10 |
| *Aspergillus turbingensis* | 0 | 10 |
| *Fusarium species* | 30 | 40 |
| *Penicillium species* | 0 | 10 |
| *Gliocladium cibotti* | 0 | 30 |
| *Pichia kudriavzevii* | 40 | 20 |
| *Syncephalastrum racemosum* | 0 | 10 |
| *Rhizopus delemar* | 30 | 30 |

**Table 4.13 Indices of similarity of location with respect to occurrence of mycotoxigenic fungi in tea**

| Location/Product | a | b | c | Index of similarity |
|---|---|---|---|---|
| Mambilla green-Mambilla black | 4 | 5 | 3 | 66.7 |
| Mambilla green-Ibadan green | 4 | 2 | 1 | 33.3 |
| Mambilla green-Ibadan black | 4 | 3 | 1 | 28.6 |
| Mambilla black-Ibadan green | 5 | 2 | 0 | 0 |
| Mambilla black-Ibadan black | 5 | 3 | 0 | 0 |
| Ibadan green-Ibadan black | 2 | 3 | 2 | 80.0 |

a- Number of mycotoxigenic species at one location/product;

b- Number of mycotoxigenic species at the other location/product;

c- Number of mycotoxigenic species common to ‘a and b’.

Index of Similarity, S=2c/a+b x 100 (Aroyeun *et al*. 2011)

similarity of 33.3% and 28.6% respectively. Index of similarity between Mambilla black tea and Ibadan green tea like Mambilla black tea and Ibadan black teas is zero (Table 4.13). Strong similarity again exists between Ibadan green tea and Ibadan black tea (80%). This relationship indicates the influence of location and processing on fungi contamination of food products. Observed result corroborates the position of Hernandez and Martinez (2018), on environmental impact of fungi and products handling at every stage of production. Sample with Index of similarity above 50%, indicates they might have originated from same source or region since they were collected from trade.

### 4.5.1 Mycobiota of coffee from Ibadan and Mambilla

Coffee, like other beverage crops, undergoes heavy microbial infestation during storage activities. Table 4.14 shows various fungal species found in coffee. *Aspergillus* species was dominant (Table 4.14, 4.15, 4.16), although, *Aspergillus* species and mould were identified. Green coffee from Ibadan had *Penicillium georgiense* and *Aspergillus foetidus*. Green coffee from Mayo-selbe, in addition to the fungal flora detected, had *Rizopus delemar* isolated from it. Fungi isolated from Mambilla green coffee were mainly *Aspergillus niger*. Black coffee from Idi Ayunre was laced with *Pichia kudriavzevii*, a mould, while *Aspergillus niger*, and P*enicillium* species were found in black coffee from Mayo-selbe and Kusuku (Mambilla) respectively. Table 4.16 shows the prevalence of contamination in coffee. *Aspergillus niger* in green coffee was 25% and 75% infestation level in black coffee. Also, *Penicillium* species in green coffee was 25% and 50% in black coffee. This was in agreement with previous studies on contamination of coffee by Ochratoxins (UNCTAD, 2005), and its mitigation in coffee beverages. Comparing different warehouses (Table 4.17) using species composition showed that the sites most similar in their fungal flora over the sampling period were Idi-Ayunre- Mayoselbe and Idi-Ayunre- Kusuku. Index of similarity above 50% indicates effect of environment on storage of these commodities. This result contradicts claims by Veloso *et al* (2020), that pattern does not exist in organisms obtained in different regions which represents the influence of edapho-climatic conditions on the microbial communities.

**Table 4.14 Mycotoxigenic fungi detected in coffee from Ibadan, Oyo state, Nigeria**

| Coffee | Fungi detected |
|---|---|
| IGCc**2** | *Penicillium georgiense, Aspergillus foetidus,* |
| IBCc3 | *Aspergillus tubingensis, Aspergillus niger* |

**Table 4.15 Mycotoxigenic fungi detected in coffee from Mambilla, Taraba state, Nigeria**

| Coffee | Fungi detected |
|---|---|
| MGCc**1** | *Aspergillus tubingensis* |
| MBCc3 | *Aspergillus niger* |
| MGCc2 | *Aspergillus niger, Aspergillus foetidus* |
| MBCc**4** | *Aspergillus niger, Aspergillus foetidus Penicillium specie* |

**Table 4.16 Percentage occurrences of fungi and mould in coffee**

| Fungi | Green coffee (%) | Black coffee (%) |
|---|---|---|
| *Aspergillus niger* | 25 | 75 |
| *Aspergillus foetidus* | 50 | 25 |
| *Aspergillus turbingensis* | 25 | 25 |
| *Penicillium specie* | 25 | 50 |
| *Pichia kudriavzevii* | 0 | 25 |

**Table 4.17 Indices of similarity of location with respect to occurrence of mycotoxigenic fungi in coffee**

| Location | a | b | c | Index of similarity |
|---|---|---|---|---|
| Idi Ayunre-Mayoslebe | 4 | 2 | 2 | 66.7 |
| Idi Ayunre-Kusuku | 4 | 5 | 3 | 66.7 |
| Mayosebe-Kusuku | 2 | 5 | 1 | 28.6 |

a- Number of mycotoxigenic species at one location/product;

b- Number of mycotoxigenic species at the other location/product;

c- Number of mycotoxigenic species common to 'a and b'.

Index of Similarity, S=2c/a+b x 100 (Aroyeun *et al*., 2011)

**Table 4.18: Aspergillus Toxin in Tea**

| Parameters | Variety mean (µg/kg) | | p- value | Location mean (µg/kg) | | p-value |
|---|---|---|---|---|---|---|
| | Green tea | Black tea | | Ibadan | Mambilla | |
| A. Aflatoxin $B_1$ | 0.000 ± 0.00 | 478.40 ± 87.24 | 0.009** | 93.50 ± 28.78 | 384.90 ± 86.83 | 0.306 |
| A. Aspergillimide | 0.360 ± 0.11 | 2.900 ± 0.89 | 0.937 | 0.000 ± 0.00 | 3.260 ± 0.89 | 0.004** |
| A. Aspinolid B | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Averantin | 14.30 ± 0.29 | 2.880 ± 0.61 | 0.771 | 3.570 ± 0.61 | 0.730± 0.23 | 0.108 |
| A. Bisgliotoxin | 0.000 ± 0.00 | 7.990 ± 2.46 | 0.162 | 0.000 ± 0.00 | 7.990 ± 2.46 | 0.162 |
| A. Fumigaclavine | 0.000 ± 0.00 | 12.260 ± 3.77 | 0.162 | 0.000 ± 0.00 | 12.260 ± 3.77 | 0.162 |
| A. Fumigaclavine C | 14.44 ± 4.45 | 499.390±132.33 | 0.306 | 0.000 ± 0.00 | 513.830±131.83 | 0.009** |
| A. Fumiquinazolin A | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Fumiquinazolin D | 6.330 ± 1.95 | 234.50 ± 63.87 | 0.306 | 0.000 ± 0.00 | 240.82 ± 63.66 | 0.009** |
| A. Fumitremorgin C | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Integracin B | 0.000 ± 0.00 | 0.630 ± 0.19 | 0.162 | 0.000 ± 0.00 | 0.630 ± 0.19 | NA |
| A. Kotanin A | 1.310 ± 0.40 | 1.630± 0.50 | 0.937 | 0.000 ± 0.00 | 2.940 ± 0.61 | 0.04* |
| A. Methylsulochrin | 0.000 ± 0.00 | 28.930 ± 8.90 | 0.162 | 0.000 ± 0.00 | 28.930 ± 8.90 | 0.162 |
| A. O-Methylsterigmatocystin | 0.000 ± 0.00 | 2.640 ± 0.59 | 0.04* | 0.000 ± 0.00 | 2.640 ± 0.59 | 0.04* |
| A. Phenopyrrozin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Pseurotin A | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Pyripyropene A | 0.000 ± 0.00 | 30.300 ± 8.00 | 0.04* | 0.000 ± 0.00 | 30.300 ± 8.00 | 0.04* |
| A. Pyripyropene D | 0.000 ± 0.00 | 3.880 ± 0.99 | 0.04* | 0.000 ± 0.00 | 3.880 ± 0.99 | 0.04* |
| A. seco-Sterigmacystin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Sterigmacystin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Trypacidin | 0.000 ± 0.00 | 4.780 ± 1.47 | 0.162 | 0.000 ± 0.00 | 4.780 ± 1.47 | 0.162 |
| A. Tryprostatin B | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Tryptoquivaline F | 0.000 ± 0.00 | 306.04 ± 83.53 | 0.04* | 0.000 ± 0.00 | 306.04 ± 83.53 | 0.04* |

NA- Not Applicable, alpha (p-value) level- 0.05.

### 4.6.0 Aspergillus toxins in green tea and black tea

Generally, about twenty-three (23) Aspergillus toxins were quantified in both green tea and black tea. Table 4.18 shows the result of quantitation of various Aspergillus toxins present in green teas and black teas, and the contribution of originating location, that is; Ibadan and Mambilla respectively. $AfB_1$ was obviously a concern as found in the results with significant presence in black teas. The alpha level (p-value) for $AfB_1$ present in black teas analysed was 0.009. This is an indication of the likelihood of aflatoxin $B_1$, being a problem in black tea varieties. The contributions of the originating locations were also assessed and this revealed that location had negligible impact on $AfB_1$ contamination in black tea varieties. This result thus agrees with Hernandez and Martinez (2018), who argued that beyond contamination on the field, mycotoxins presence could also emanated during transportation, handling and storage of black tea commodities. Similarly, quantitation of aspergillimide and fumigaclavin C toxins revealed their presence in both green teas and black teas. Although these toxins were detected and quantified, they were not significant ($P > 0.05$) to be a problem. Meanwhile, the contribution of the originating location of these tea commodities was significant. Table 4.18 showed that Mambilla has a statistically significant impact on aspergillimide and fumigaclavin C toxins in tea varieties assessed with an alpha level of 0.004 and 0.009 respectively. This result also agrees with fumiquinazolin D and kotanin A toxins. O- Methylsterigmatocystin (2.640 µg/kg), pyripyropene A (30.3 µg/kg), pyripyropene D (3.88 µg/kg) and tryptoquivalin F (306.04 µg/kg) toxins were significantly present ($p < 0.05$) in black tea commodities from Mambilla. These results substantially agree with Haque *et al*. (2020), who argued that cultural practices, processing and storage facilities employed in developing countries were inadequate to stem problem of mycotoxins contamination in food commodities. Aflatoxin $B_1$ ($AfB_1$) from *Aspergillus flavus* was found in high quantity in black tea commodities from Ibadan and Mambilla. From Table 4.30, quantified $AfB_1$ was as high as 271.2, 113.8, and 93.5 µg/kg respectively in MC**5**B, MC**2**B and IC**2**B. $AfB_1$ contamination level in these black tea commodities were much higher when compared to E.U regulatory limit of 2- 8 µg/kg in food items (Aiko and Mehta, 2015).

### 4.6.1 Penicillium toxins in green and black tea

About sixteen *Penicillium* toxins were quantified in tea commodities obtained from Ibadan and Mambilla respectively. Table 4.19 presents the level of *penicillium* contamination in green tea and black tea. Some of the toxins include, Atpenin A5, Chaetominine, Chanoclavin, Citrinin, Cyclopeptin, Flavoglaucin, e.t.c. From Table 4.19, Flavoglaucin (11.07 µg/kg), Oxaline (0.08 µg/kg), PF1163A (4.92 µg/kg) and Quinolactacin A (1.85 µg/kg) toxins were quantified in green tea from Mambilla in Taraba State. Over all, the significant metabolites are oxaline (p= 0.04), PF1163A (p= 0.04) and quinolactacin A (p= 0.003).

**Table 4.19: Penicillium Toxins in Tea**

| Parameters | Variety mean (µg/kg) | | p-value | Location mean (µg/kg) | | p- value |
|---|---|---|---|---|---|---|
| | Green tea | Black tea | | Ibadan | Mambilla | |
| P. Atpenin A5 | 0.000 ± 0.00 | 1.00 ± 0.31 | 0.162 | 0.000 ± 0.00 | 1.00 ± 0.31 | 0.162 |
| P. Chaetominine | 0.000 ± 0.00 | 92.03 ± 28.33 | 0.162 | 0.000 ± 0.00 | 92.03 ± 28.33 | 0.162 |
| P. Chanoclavin | 0.000 ± 0.00 | 2.43 ± 0.75 | 0.162 | 0.000 ± 0.00 | 2.43 ± 0.75 | 0.162 |
| P. Citrinin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| P. Cyclopeptine | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| P. Dechlorogriseofulvin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| P. Dihydrocitrinone | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| P. Flavoglaucin | 11.07 ± 3.41 | 22.17 ± 4.19 | 0.146 | 30.51 ± 5.01 | 2.73 ± 0.84 | 0.078 |
| P. Griseofulvin | 0.000 ± 0.00 | 1.23 ± 0.38 | 0.162 | 0.000 ± 0.00 | 1.23 ± 0.38 | 0.162 |
| P. Notoamide Derivative | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| P. NP1866 | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| P. Oxaline | 0.08 ± 0.02 | 2.35 ± 0.72 | 0.937 | 0.000 ± 0.00 | 2.43± 0.72 | 0.04* |
| P. PF1163A | 4.92 ± 1.51 | 6.43 ± 1.98 | 0.937 | 0.000 ± 0.00 | 11.35 ± 2.36 | 0.04* |
| P. Preussin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| P. Quinolactacin A | 1.85 ± 0.37 | 0.88 ± 0.15 | 0.751 | 0.14 ± 0.04 | 2.58 ± 0.36 | 0.003** |
| P. Quinolactacin B | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |

* NA- Not Applicable, alpha (p-value) level- 0.05

### 4.6.2 Fusarium toxins in green tea and black tea

*Fusarium* toxins quantified in tea commodities were eleven (11) in all and Table 4.20 presents the various toxin contaminants in green tea and black tea. For green tea commodities, beauvericin and fusarenon-X toxins were found to have a mean value of 0.68 µg/kg and 200.02 µg/kg while other toxins were not detected (below limit of detection). *Fusarium* species implicated solely for this were *Fusarium solani*, *Fusarium nivale* and *Fusarium* spp. (Bhattacharyya, *et al*, 2016). Table 4.20 also showed the level of contamination of deoxynivalenol, beauvericin, fusarenon-X and gibberellin A12 toxins in black tea commodities. The increasing quantity of *Fusarium* toxins found in tea commodities, ranging from green tea to black tea, calls for action. Although, European Union regulatory limit for DON and allied toxins specified 200-700 µg/kg in food raw materials intended as input for further production processes, yet FDA advised maximum of 1 ppm (1 µg/g) for food items meant for direct consumption. Deoxynivalenol toxins (DON) was quite high when FDA's advisory is compared with results found in certain black teas as presented on Table 4.31. This implies an increase on the minimum of about thirty percent (30 %) and on the maximum of about five hundred percent (500%) above FDA's advisory on level of DON in foods for direct consumption (European commission, 2017).

### 4.6.3 Other fungi toxins in green tea and black tea

Table 4.21 showed amounts of other fungi toxins higher than most toxins quantified in this work. Abscisic acid, unspecific brevianimide F, unspecific cyclo (L-pro-L-Tyr) and unspecific rugulusovin were traceable to activities of other fungi present either in green

**Table 4.20: Fusarium Toxins in Tea**

| Parameters | Variety mean (µg/kg) | | p- value | Location mean (µg/kg) | | p- value |
|---|---|---|---|---|---|---|
| | Green tea | Black tea | | Ibadan | Mambilla | |
| F. DON | 0.000 ± 0.00 | 17.78 ± 1.57 | 0.001** | 4.69 ± 0.97 | 13.09 ± 1.68 | 0.059 |
| F. alpha-Zearalenol | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| F. Aurofusarin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| F. Beauvericin | 0.68 ± 0.12 | 3.47 ± 0.94 | 0.367 | 0.05± 0.02 | 4.09 ± 0.93 | 0.0001** |
| F. Bikaverin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| F. Deoxygerfelin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| F. Fusarenon-X | 200.02 ± 33.68 | 148.05 ± 45.57 | 0.194 | 35.70 ± 10.99 | 313.27 ± 51.98 | 0.078 |
| F. Fusaric acid | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| F. Gibberellin A12 | 0.000 ± 0.00 | 78.94 ± 24.30 | 0.162 | 0.000 ± 0.00 | 78.94 ± 24.30 | 0.162 |
| F. Sambutoxin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| F. W493 | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |

* NA- Not Applicable, alpha (p-value) level- 0.05

**Table 4.21: Other Fungal Toxins in Tea**

| Parameters | Variety mean (µg/kg) | | p- value | Location mean (µg/kg) | | p- value |
|---|---|---|---|---|---|---|
| | Green tea | Black tea | | Ibadan | Mambilla | |
| Abscisic acid | 18331.20 ± 2925.06 | 0.000 ± 0.00 | 0.009** | 0.000 ± 0.00 | 18331.20± 2925.06 | 0.01** |
| Bassianolide | 0.000 ± 0.00 | 0.19 ± 0.06 | 0.162 | 0.000 ± 0.00 | 0.19 ± 0.06 | 0.162 |
| Cylindrol B | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Ilicicolin B | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| LL-Z1272e | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Phomalone | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Sporidesmolide 11 | 7.29 ± 0.88 | 10.77 ± 0.81 | 0.166 | 2.24 ± 0.38 | 15.82 ± 0.61 | <0.001*** |
| Alternaria Alternariomethylether | 0.000 ± 0.00 | 1.21 ± 0.37 | 0.162 | 0.000 ± 0.00 | 1.21 ± 0.37 | 0.162 |
| Unspecific Asperglaucide | 2025.18 ± 398.77 | 5590.15± 1083.65 | 0.818 | 2017.60 ± 621.00 | 5597.73 ± 973.19 | 0.001** |
| Unspecific Asperphenamate | 28683.36 ± 3517.51 | 14656.85 ± 2323.08 | 0.966 | 11715.31± 2409.34 | 31624.90± 3303.61 | 0.002** |
| Unspecific Brevianamid F | 33.64 ± 4.50 | 106.23 ± 9.89 | 0.013** | 12.43 ± 2.61 | 127.43± 8.33 | <0.001** |
| Unspecific cyclo (L-pro-L-Tyr) | 147.81 ± 18.97 | 446.48 ± 43.09 | 0.017** | 50.43 ± 10.55 | 543.86± 35.96 | <0.001*** |
| Unspecific cyclo (L-pro-L-Val) | 76.76 ± 9.19 | 254.65 ± 30.68 | 0.143 | 28.54 ± 5.46 | 302.88± 27.68 | <0.001*** |
| Unspecific Emodin | 5.81 ± 1.38 | 42.63 ± 8.17 | 0.085 | 0.97 ± 0.28 | 47.46 ± 8.02 | <0.001*** |
| Unspecific Endocrocin | 0.000 ± 0.00 | 102.74 ± 31.62 | 0.162 | 0.000 ± 0.00 | 102.74 ± 31.62 | 0.162 |
| Unspecific Fallacinol | 0.000 ± 0.00 | 5.10 ± 1.57 | 0.162 | 0.000 ± 0.00 | 5.10 ± 1.57 | 0.162 |
| Unspecific Iso-Rhodoptilometrin | 0.77 ± 0.24 | 2.93 ± 0.71 | 0.395 | 1.42 ± 0.29 | 2.27 ± 0.70 | 0.499 |
| Unspecific N-Benzoyl-Phenylalanine | 10902.79 ± 1160.84 | 10836.96 ± 1714.16 | 0.404 | 12275.43± 1981.67 | 9876.11± 1071.70 | 0.208 |
| Unspecific Neoechinulin A | 0.000 ± 0.00 | 0.44 ± 0.14 | 0.246 | 0.000 ± 0.00 | 0.44± 0.14 | 0.247 |
| Unspecific Norlichexanthone | 0.000 ± 0.00 | 4.36 ± 1.34 | 0.246 | 0.000 ± 0.00 | 4.36 ± 1.34 | 0.247 |
| Unspecific Orsellinic acid | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Unspecific Rugulusovin | 12.21 ± 1.10 | 0.000 ± 0.00 | 0.0001** | 0.000 ± 0.00 | 8.54 ± 1.07 | 0.008** |
| Unspecific Tryptophol | 751.96 ± 156.44 | 566.93 ± 142.42 | 0.676 | 153.61 ± 19.34 | 985.78 ± 184.17 | 0.479 |

* NA- Not Applicable, alpha (p-value) level- 0.05

tea or black tea commodities. The afore-listed toxins were present in significant quantities. The mean value of abscisic acid contaminant in green tea commodities analysed was 18331.20 µg/kg but not detected in black tea commodities. This is statistically significant as p-value was 0.009. Originating source also significantly (p=0.01) contributed to this result. Unspecific brevianimide F was 33.64 µg/kg, in green tea and 106.23 µg/kg in black tea. This was significant in all tea commodities and the source location also contributed highly to this contaminant in tea. Unspecific cyclo (L-pro-L-Tyr) and unspecific rugulusovin had similar result when compared with unspecific brevianimide F. Sporidesmolide II toxin was present in both the green teas and black teas. This toxin, sporidesmolide II, was 7.29 µg/kg in geen tea and 10.77 µg/kg in black tea commodities. Source of these tea commodities had significant ($p< 0.001$) alpha level on sporidesmolide II in teas. Unspecific asperglaucide, unspecific asperphenamate, unspecific cyclo (L-pro-L-Val) and unspecific emodin were detected and quantified in green and black teas. Table 4.19 again, showed the very significant contribution made by source location of these tea commodities to the listed toxins.

### 4.6.4 Bacteria and Plant toxins in tea

Bacteria toxins quantified in tea were mainly five. Table 4.22 revealed that green teas and black teas contain an undetectable amount of bacteria toxins. These toxins include Chloramphenicol, Monactin, Nonactin, Staurosporin and Valinomycin. The singular reason for this was as a result of low available water in tea (see Table 4.44) at variant to water needed for bacteria activity in tea. Since water was not readily available, bacteria automatically cannot thrive.

Table 4.23 showed results of plant toxins quantified in green teas and black teas. Daidzin toxin, Genistein toxin, Genistin toxin and Prunasin toxins were below limit of detection in green teas analysed. Although the alpha level for prunasin toxin in tea commodities was higher than 5 % ($p> 0.05$), reported mean value was 223.52 µg/kg in black tea obtained from Ibadan.

### 4.6.5 Toxin profile in green and black coffee

Table 4.24 shows the various *Aspergillus* toxins quantified in both green coffee and black coffee obtained from Ibadan and Mambilla. All the twenty-three (23) toxins screened in

**Table 4.22: Bacteria Toxins in Tea**

| Parameters | Variety mean (µg/kg) | | p-value | Location mean (µg/kg) | | p-value |
|---|---|---|---|---|---|---|
| | Green tea | Black tea | | Ibadan | Mambilla | |
| Bacteria Chloramphenicol | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Bacteria Monactin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Bacteria Nonactin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Bacteria Staurosporin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Bacteria Valinomycin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |

* NA- Not Applicable, alpha (p-value) level- 0.05

**Table 4.23: Plant Toxins in Tea**

| Parameters | Variety mean (µg/kg) | | p-value | Location mean (µg/kg) | | p-value |
|---|---|---|---|---|---|---|
| | Green tea | Black tea | | Ibadan | Mambilla | |
| Plants Daidzin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Plants Genistein | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Plants Genistin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Plants Prunasin | 0.000 ± 0.00 | 223.52 ± 68.80 | NA | 447.04 ± 94.24 | 0.000 ± 0.00 | 0.167 |

* NA- Not Applicable, alpha (p-value) level- 0.05

**Table 4.24: Aspergillus Toxins in Coffee**

| Parameters | Variety mean (µg/kg) Green coffee | Black coffee | p-value | Location mean (µg/kg) Ibadan | Mambilla | p-value |
|---|---|---|---|---|---|---|
| A. Aflatoxin B1 | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Aspergillimide | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Aspinolid B | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Averantin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Bisgliotoxin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Fumigaclavine | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Fumigaclavine C | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Fumiquinazolin A | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Fumiquinazolin D | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Fumitremorgin C | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Integracin B | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Kotanin A | 4.00 ± 0.51 | 13.58 ± 1.88 | 0.693 | 13.67 ± 1.88 | 3.91 ± 0.50 | 0.398 |
| A. Methylsulochrin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. O-Methylsterigmatocystin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Phenopyrrozin | 17.69 ± 3.28 | 20.09 ± 3.72 | 0.835 | 0.000 ± 0.00 | 37.78 ± 4.06 | 0.032 |
| A. Pseurotin A | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Pyripyropene A | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Pyripyropene D | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. seco-Sterigmacystin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Sterigmacystin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Trypacidin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Tryprostatin B | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| A. Tryptoquivaline F | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |

* NA- Not Applicable, alpha (p-value) level- 0.05

coffee were below limits of detection except for Kotanin A and Phenopyrrozin toxins. Kotanin A comtamination was found in both green coffee and black coffee although, its quantity in black coffee was higher than values in green coffee. Kotanin A was present in green coffee from Ibadan and Mambilla as well as in black coffee from Ibadan and Mambilla. Notwithstanding, presence of kotanin A in tea commodities assessed was not significant ($p > 0.05$) to pose quality and health issues. Phenopyrrozin toxin was present in both green and black coffees obtained from Mambilla. Mean value of 17.69 µg/kg of Phenopyrrozin toxin was reported in green coffee and 20.09 µg/kg found in black coffee. Previous studies had reported presence of Ochratoxin A, OTA, in coffee (Demelash and Ashenafi, 2019). Alpha level ($p = 0.032$) was reported for phenopyrrozin toxin in tea commodities from Mambilla. This implies that source location imparts this toxin significantly in teas. Table 4.25 shows Penicillium toxins quantified in coffee. Of all sixteen (16) toxins screened, only six (6) were detected and quantified in both green and black coffee and other toxins were not detected (below limit of detection). Flavoglaucin, a *penicillium* toxin, was present in both green coffees and black coffees obtained from Ibadan and Mambilla. Also, NP1866 toxin was present in green and black coffee from Ibadan. The threshold for NP1866 toxin was higher than alpha limit ($p > 0.05$) in samples analysed. Oxaline and PF1163A were found in screened black coffee commodities. Majority of these toxins were higher than 2ppb specified for OTA in coffee (Aiko and Mehta, 2015). Quinolactacin A and Quinolactacin B were quantified in green coffee and black coffee. Their presence in these commodities were insignificant ($p > 0.05$) but there were high tendencies, ($p=0.032$), that quinolactacin B threshold could increase in commodities from Ibadan. This finding corroborates the work of Garcia-Moraleja *et al*. (2015), on coffee beverages.

Quantified *Fusarium* toxins were presented in Table 4.26. Aurofusarin toxin, Beauvericin toxin, fusaric acid and W493 toxins showed positive trend in green coffee to black coffee from same location. Aurofusarin in green coffee had a mean value of 29.72 µg/kg and in black coffee, increased to 57.28 µg/kg. Similarly, Fusaric acid in green coffee had a mean value of 358.84 µg/kg and in black coffee, was 2817.60 µg/kg. W493 toxin was 377.74

**Table 4.25: Penicillium Toxins in Coffee**

| Parameters | Variety mean (µg/kg) | | p-value | Location mean (µg/kg) | | p-value |
|---|---|---|---|---|---|---|
| | Green coffee | Black coffee | | Ibadan | Mambilla | |
| P. Atpenin A5 | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| P. Chaetominine | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| P. Chanoclavin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| P. Citrinin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| P. Cyclopeptine | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| P. Dechlorogriseofulvin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| P. Dihydrocitrinone | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| P. Flavoglaucin | 238.70 ± 19.11 | 143.37± 18.11 | NA | 311.14 ± 20.17 | 70.93 ± 2.89 | NA |
| P. Griseofulvin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| P. Notoamide Derivative | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| P. NP1866 | 28.44 ± 5.27 | 6.69 ± 1.24 | 0.835 | 35.12 ± 4.99 | 0.000 ± 0.00 | 0.032 |
| P. Oxaline | 0.000 ± 0.00 | 3.14 ± 0.49 | 0.111 | 2.36± 0.44 | 0.000 ± 0.00 | 0.243 |
| P. PF1163A | 0.000 ± 0.00 | 0.44± 0.08 | 0.169 | 0.000 ± 0.00 | 0.44 ± 0.08 | 0.169 |
| P. Preussin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| P. Quinolactacin A | 3.94 ± 0.52 | 12.22± 1.92 | 1 | 13.87± 1.87 | 2.29 ± 0.26 | 0.213 |
| P. Quinolactacin B | 0.12 ± 0.02 | 0.62± 0.12 | 0.835 | 0.74 ± 0.11 | 0.000 ± 0.00 | 0.032 |

* NA- Not Applicable, alpha (p-value) level- 0.05

**Table 4.26: Fusarium Toxins in Coffee**

| Parameters | Variety mean (µg/kg) | | p-value | Location mean (µg/kg) | | p-value |
|---|---|---|---|---|---|---|
| | Green coffee | Black coffee | | Ibadan | Mambilla | |
| F. DON | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| F. alpha-Zearalenol | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| F. Aurofusarin | 29.72 ± 3.27 | 57.28 ± 6.14 | 0.398 | 0.000 ± 0.00 | 87.00 ± 3.08 | 0.0003 |
| F. Beauvericin | 1.44 ± 0.20 | 2.34 ± 0.38 | 1 | 0.51 ± 0.06 | 3.27 ± 0.38 | 0.399 |
| F. Bikaverin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| F. Deoxygerfelin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| F. Fusarenon-X | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| F. Fusaric acid | 358.84± 66.44 | 2817.60 ± 0.00 | 0.835 | 0.000 ± 0.00 | 2676.44 ± 411.78 | 0.032 |
| F. Gibberellin A12 | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| F. Sambutoxin | 0.000 ± 0.00 | 0.63 ± 0.12 | 0.169 | 0.000 ± 0.00 | 0.63 ± 0.12 | 0.17 |
| F. W493 | 377.74 ± 032.04 | 564.28 ± 47.79 | 0.313 | 117.08 ± 12.71 | 824.92 ± 22.01 | 0.001 |

* NA- Not Applicable, alpha (p-value) level- 0.05

**Table 4.27: Other Fungal Toxins in Coffee**

| Parameters | Variety mean (µg/kg) | | p-value | Location mean (µg/kg) | | p-value |
|---|---|---|---|---|---|---|
| | Green coffee | Black coffee | | Ibadan | Mambilla | |
| Abscisic acid | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Bassianolide | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Cylindrol B | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Ilicicolin B | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| LL-Z1272e | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Phomalone | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Sporidesmolide 11 | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Alternaria Alternariomethylether | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Unspecific Asperglaucide | 311.59 ± 9.69 | 220.92 ± 19.07 | 0.101 | 327.16 ± 13.87 | 205.32 ± 15.08 | 0.101 |
| Unspecific Asperphenamate | 35.32 ± 3.13 | 103.75 ± 15.45 | 0.873 | 112.08 ± 14.89 | 26.99 ± 3.49 | 0.099 |
| Unspecific Brevianamid F | 12.64 ± 2.34 | 169.88 ± 18.76 | 0.163 | 113.08 ± 17.59 | 69.44 ± 12.86 | 0.364 |
| Unspecific cyclo (L-pro-L-Tyr) | 5356.20± 219.41 | 18340.00 ±1348.22 | 0.101 | 16806.40± 383.61 | 7509.80 ± 696.11 | 0.224 |
| Unspecific cyclo (L-pro-L-Val) | 1511.70 ± 168.66 | 3121.24 ± 340.87 | 0.428 | 4043.28 ± 299.39 | 589.66 ± 40.62 | 0.04* |
| Unspecific Emodin | 0.96 ± 0.11 | 0.89 ± 0.16 | 0.671 | 1.85 ± 0.14 | 0.000 ± 0.00 | 0.004** |
| Unspecific Endocrocin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Unspecific Fallacinol | 3.36 ± 0.62 | 0.000 ± 0.00 | 0.169 | 3.36 ± 0.62 | 0.000 ± 0.00 | 0.169 |
| Unspecific Iso-Rhodoptilometrin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Unspecific N-Benzoyl-Phenylalanine | 205.76 ± 14.34 | 57.61 ± 5.28 | 0.062 | 191.78 ± 12.84 | 71.60 ± 10.56 | 0.038* |
| Unspecific Neoechinulin A | 21.23 ± 3.93 | 21.02 ± 3.89 | 0.835 | 42.25 ± 4.52 | 0.000 ± 0.00 | 0.032* |
| Unspecific Norlichexanthone | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Unspecific Orsellinic acid | 1014.863 ± 427.97 | 1571.975 ± 394.88 | 0.039* | 1479.880± 307.18 | 1106.958±585.33 | 0.102 |
| Unspecific Rugulusovin | 145.81 ± 11.71 | 173.40 ± 14.56 | 1.000 | 257.31 ± 10.94 | 61.90 ± 3.94 | 0.004** |
| Unspecific Tryptophol | 92.295 ± 44.81 | 115.792 ± 70.97 | 0.428 | 136.828 ± 50.02 | 71.259 ± 46.87 | 0.013** |

* NA- Not Applicable, alpha (p-value) level- 0.05

µg/kg in green coffee and a variety process mean value of 564.28 µg/kg in black coffee. This increased trend could be noticed in coffee commodities from Mambilla except for W493 present significantly in commodities from both Ibadan and Mambilla. Contaminants like alpha-Zearalenol, bikaverin, deoxygerfelin, fusarenon-X and gibberellin A12 were not detected in all the coffee commodities screened.

Table 4.27 presents a list of other fungi toxins in coffee from Ibadan and Mambilla. About eleven (11) out of the twenty-three (23) toxins quantified were below limit of detection (not detected). Unspecific asperglaucide, unspecific asperphenamate, unspecific brevianamid F, unspecific cyclo (L-pro-L-Tyr), unspecific cyclo (L-pro-L-Val), emodin, fallacinol e.t.c, were present in green coffee and black coffee. As observed with fusarium toxins, majority of these other fungi toxins (Table 4.26) also showed a positive increasing trend from green coffee to black coffee and along location from which they were sourced. Apart from a decreased trend noticed with unspecific asperglaucide in green coffee to black coffee, unspecific emodin, unspecific fallacinol, unspecific N-benzoylphenylalanine and unspecific neoechinulin A all decreased in quantity whereas, unspecific asperphenamate, unspecific brevianimide F, unspecific cyclo (L-pro-L-Tyr), unspecific cyclo (L-pro-L-Val), unspecific orsellinic acid, unspecific rugulusovin and unspecific tryptophol all increased from green coffee to black coffee. Alpha level of unspecific orsellinic acid toxin was significant ($p< 0.05$) both in green and black coffee.

Table 4.28 showed that Chloramphenicol, Monactin, Nonactin, Staurosporin and Valinomycin toxins were not detected in coffee. This result was expected due to low available water in coffee, which does not support bacteria activity.

Plant toxins in green and black coffee were presented on Table 4.29. Daidzin, Genistein, Genistin and Prunasin toxins were quantified. Prunasin was not detected in coffee samples. Daidzin toxin and Genistin toxins showed positive increasing trend except for Genistein which decreased from 741.31 µg/kg in green coffee to 268.05 µg/kg in black tea sourced from Ibadan. Emphatically, only coffee obtained from Ibadan had higher amount of ($p= 0.032$) listed plant toxins, an indication impact of source location while toxins in coffee from Mambilla were below the limit of detection. All these plant materials quantified in coffee were not mentioned by Singh *et al*. (2013) when he reported

**Table 4.28: Bacteria Toxins in Coffee**

| Parameters | Variety mean (µg/kg) | | p-value | Location mean (µg/kg) | | p-value |
|---|---|---|---|---|---|---|
| | Green coffee | Black coffee | | Ibadan | Mambilla | |
| Bacteria Chloramphenicol | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Bacteria Monactin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Bacteria Nonactin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Bacteria Staurosporin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |
| Bacteria Valinomycin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |

* NA- Not Applicable, alpha (p-value) level- 0.05

**Table 4.29: Plant Toxins in Coffee**

| Parameters | Variety (µg/kg) | | p-value | Location mean (µg/kg) | | p-value |
|---|---|---|---|---|---|---|
| | Green coffee | Black coffee | | Ibadan | Mambilla | |
| Plants Daidzin | 31.92 ± 5.91 | 83.57 ± 15.48 | 0.835 | 115.49±14.61 | 0.000±0.00 | 0.032 |
| Plants Genistein | 741.31± 137.26 | 268.05 ± 49.63 | 0.835 | 1009.36± 129.47 | 0.000 ± 0.00 | 0.032 |
| Plants Genistin | 52.08 ± 9.64 | 227.68± 42.16 | 0.835 | 279.76± 39.99 | 0.000 ± 0.00 | 0.032 |
| Plants Prunasin | 0.000 ± 0.00 | 0.000 ± 0.00 | NA | 0.000 ± 0.00 | 0.000 ± 0.00 | NA |

* NA- Not Applicable, alpha (p-value) level- 0.05

**Table 4.30 Quantitation of regulated Aflatoxin $B_1$ ($AfB_1$) in tea**

| Sample | Variety | EU Limit (µg/kg) | AfB1 (µg/kg) |
|---|---|---|---|
| MC5B | Black tea | 2 - 8 | $271.2 \pm 156.58^a$ |
| MC3B | Black tea | 2 - 8 | ND |
| MC8G | Green tea | 2 – 8 | ND |
| MC2B | Black tea | 2 - 8 | $113.8 \pm 11.72^b$ |
| MC7G | Green tea | 2 - 8 | ND |
| MC4B | Black tea | 2 - 8 | ND |
| MC9G | Green tea | 2 - 8 | ND |
| MC1B | Black tea | 2 - 8 | ND |
| MC6G | Green tea | 2 - 8 | ND |
| IC2B | Black tea | 2 - 8 | $93.5 \pm 8.23^c$ |
| IC4G | Green tea | 2 - 8 | ND |
| IC1B | Black tea | 2 - 8 | ND |
| IC3G | Green tea | 2 - 8 | ND |
| MCIGK | Green tea | 2 - 8 | ND |

ND – Not Detected, superscrip ‘a, b, c’ show sample significant different ($P<0.05$) from other samples along same vertical column

**Table 4.31 Quantitation of deoxynivalenol toxin (DON) in tea**

| Sample | Variety | FDA Advisory Limit (µg/kg) | DON (µg/kg) |
|---|---|---|---|
| MC5B | Black tea | ≤1.0 | 5.18[a] |
| MC3B | Black tea | ≤1.0 | 1.30[e] |
| MC8G | Green tea | ≤1.0 | ND |
| MC2B | Black tea | ≤1.0 | 1.50[e] |
| MC7G | Green tea | ≤1.0 | ND |
| MC4B | Black tea | ≤1.0 | 2.81[b] |
| MC9G | Green tea | ≤1.0 | ND |
| MC1B | Black tea | ≤1.0 | 2.30[cd] |
| MC6G | Green tea | ≤1.0 | ND |
| IC2B | Black tea | ≤1.0 | 2.50[c] |
| IC4G | Green tea | ≤1.0 | ND |
| IC1B | Black tea | ≤1.0 | 2.19[d] |
| IC3G | Green tea | ≤1.0 | ND |
| MCIGK | Green tea | ≤1.0 | ND |

ND – Not Detected, superscrip ‘a, b, c, d, cd, e’ show sample significant different ($P<0.05$) from other samples along same vertical column

thirty-eight different toxins found in plants. In-depth information about these plant toxins will enhance balanced knowledge on new products development in foods.

### 4.7.0 Concentration of $AfB_1$ and DON in tea

The result presented in Table 4.30 shows that aflatoxin $B_1$ was present in commercial black tea sample at levels higher than 2-8 µg/kg specified on European unions' limit and 20 µg/kg stipulated in US FDA standard for all foods. It is worthy of note, that aflatoxin $B_1$ quantified in green tea was below limits of detection, that is, not detected (ND). This alarming figure of aflatoxin $B_1$ in black tea can be hinged on poor production practices, that is, lack of good manufacturing practices (GMP). As good as campaign for good agricultural practices may appear, it will be in-adequate if good manufacturing practices is not in place. Need to train farmers and processors at frequent intervals cannot be over emphasized. Drying of tea to very low moisture content, as seen on Table 4.44, Table 4.46 and Table 4.47; will ensure the shelf stability of the product hence limiting the growth and proliferation of aflatoxigenic fungi in tea.

Storage condition which the retailed black tea may have been subjected before bringing it to custody for analysis may contribute to poor quality. Although, low incidence of aflatoxin was found (less than 5µg/mL) in geen tea commodities, possibilities of higher quantity are imminent when there are conditions favourable to mould growth and mycotoxin production. Good hygiene practices (GHP) and avoidance of contamination during storage can help maintain the desired quality (none/low aflatoxin $B_1$) in tea and its products. Table 4.31 shows that deoxynivalenol (DON), a trichothecene, was detected in most of the tea analysed. It ranged between 1.3 µg/kg and 5.18 µg/kg in black tea commodities. This is higher than the US FDA advisory limit (1 µg/kg) of DON in foods intended for direct consumption. Certain quantity of DON was found in almost all the black teas. The green teas that were screened for deoxynivalenol contamination showed that they are free from DON. This results confirms the wide belief that green teas are safe for direct use.

### 4.7.1 Reduction of aflatoxin-$B_1$ in spiked tea treated with powder of C*urcuma longa*, X*ylopia aethiopica* and P*iper guineense*

Table 4.32 shows the effectiveness of selected tropical spices in reduction of aflatoxin $B_1$ contamination in tea. Powder extracts of *Curcuma longa* and *Piper guineense* showed high effectiveness (>92%) in reducing $AfB_1$ at all concentration levels. 1000ppm of *Xylopia aethiopica* performed close to *C. longa* and *P. guineense*. 3000ppm and 4000ppm were less effective (Table 4.32). This finding revealed that aflatoxin $B_1$ incidence in tea, can be reduced using these selected spices. It can also be deduced that smaller concentration of *Xylopia aethiopica* spice promises to be more effective in reducing aflatoxin $B_1$ in contaminated tea. This result, however, agreed with the findings of Aroyeun *et al.* (2011), who reported the use of antioxidants in the reduction of aflatoxin biosynthesis and *Aframomum danielli* oils and aqueous extracts in reduction of OTA in cocoa and cocoa products respectively.

In Table 4.33, reduction efficiency of these selected tropical spices (*C. longa, X. aethiopica* and *P. guineense*) was equally examined on deoxynivalenol (DON). Result shows that DON contaminated tea treated with different concentration of powder extract of these spices reduced the contamination. The percentage reduction efficiency of DON increased with increased concentration. This was the case with *Curcuma longer* and *Piper guineense* whereas, for *xylopia*

**Table 4.32 Effects of *Curcuma longa, Xylopia aethiopica* and *Piper quineense* treatments on aflatoxin $B_1$ ($AfB_1$) reduction in spiked tea stored for six months**

| Spice (powder) | Concentration (µg/g) | Spiked tea (ppb) | AfB1 (µg/kg)* | % RE |
|---|---|---|---|---|
| *Curcuma longa* | 1000 | 5 | ND | > 92.0 |
| | 2000 | 5 | - | - |
| | 3000 | 5 | ND | > 92.0 |
| | 4000 | 5 | ND | > 92.0 |
| *Xylopia aethiopica* | 1000 | 5 | 0.43696 | 91.3 |
| | 2000 | 5 | - | - |
| | 3000 | 5 | 1.01392 | 79.7 |
| | 4000 | 5 | 1.01216 | 79.8 |
| *Piper guineense* | 1000 | 5 | ND | > 92.0 |
| | 2000 | 5 | - | - |
| | 3000 | 5 | ND | > 92.0 |
| | 4000 | 5 | ND | > 92.0 |

*ND – Not Detected (<0.05 x $10^{-3}$µg/g), *RE- reduction efficiency - values obtained after treatment with powder extracts of selected spices.

**Table 4.33 Effects of *Curcuma longa, Xylopia aethiopica* and *Piper quineense* treatments on deoxynivalenol (DON) reduction in spiked tea stored for six months**

| Spice (Powder) | Concentration (µg/g) | Spiked tea (5 ppb) | DON (µg/kg)* | % RE |
|---|---|---|---|---|
| *Curcuma longa* | 1000 | 5 | 2.97 | 40.6 |
| | 2000 | 5 | - | - |
| | 3000 | 5 | 2.50 | 50.0 |
| | 4000 | 5 | 2.34 | 53.2 |
| *Xylopia aethiopica* | 1000 | 5 | 3.11 | 37.8 |
| | 2000 | 5 | - | - |
| | 3000 | 5 | 3.45 | 31.0 |
| | 4000 | 5 | 3.74 | 25.2 |
| *Piper guineense* | 1000 | 5 | 3.80 | 24.0 |
| | 2000 | 5 | - | - |
| | 3000 | 5 | 3.57 | 28.6 |
| | 4000 | 5 | 3.18 | 36.4 |

*RE- reduction efficiency. *- values obtained after treatment with spice powder extract,

*aethiopica* spice, the higher the concentration of the spice addition, the lower the percentage efficiency of the spice. It is convenient to submit therefore, that *Xylopia aethiopica* spice is much more effective when added in smaller quantity. Hence in terms of effectiveness, *Xylopia aethiopica* was more effective than *Curcuma longa* and the least effective was *Piper guineense*.

### 4.7.2 Combination effect of available water (aw), temperature and selected spices on production of Aflatoxin $B_1$ and DON in tea

Table 4.34 shows the effect of crude powder of *Curcuma longa* spice, $a_w$ and temperature on production of Aflatoxin $B_1$ and DON in tea. On the addition of 1000 µg/g *C. longa* spice, available water of 0.75 and temperature of 20 $^oC$, no $AfB_1$ was found in the sample. A similar result was obtained when 2000 µg/g of the same spice extract was added at constant available water but varied temperature of 25 $^oC$. About 835 pg/g $AfB_1$ was produced in tea under given conditions of 30 $^oC$, temperature and 3000 µg/g *Curcuma longa* spice. A further increase in production of $AfB_1$ to 1010 pg/g was recorded at temperature of 35 $^oC$ and spice addition level of 4000 µg/g. The observed interaction was similar at $a_w$ of 0.76, temperature of 20 $^oC$ and *C. longa* spice addition level of 1000 µg/g with the earlier result obtained under conditions 0.75 $a_w$, temperature of 20 $^oC$ and spice addition level of 1000 µg/g.

Production of $AfB_1$ was favourable at available water, $a_w$, of 0.75, optimum temperature of 30 $^oC$ and 35 $^oC$ and a spice addition of 3000 µg/g and 4000 µg/g. This observation was likewise similar given same optimum temperature and quantity of added *C. longa* and at water activity of 0.76, 0.77 and 0.78. Thus, it can be deduced that $AfB_1$ production can be minimized or prevented when temperature is maintained at 20 $^oC$ or 25 $^oC$. Also, less quantity of *C. longa* spice will be required when these factors are combined optimally to inhibit growth of fungi and production of $AfB_1$. It can be concluded that *C. longa* spice has potential to mitigate growth of *A. flavus* and production of Aflatoxin $B_1$ in tea. *C. longa* spice added at 4000 µg/g, in combination with available water of 0.76 and temperature 20 $^oC$ hindered the production of $AfB_1$. Again, Table 4.34 shows the effect of crude powder of *C. longa* spice on production of deoxynivalenol toxin in tea at specified available water and

**Table 4.34: Effect of *C. longa* spice powder, $a_w$ and temperature on the production of $AfB_1$ and DON**

| $a_w$ (%) | Temp. (°C) | *Curcuma longa* (ppm) | $AfB_1$(pg/g) | DON (ng/g) |
|---|---|---|---|---|
| 0.75 | 20 | 1000 | - | - |
| 0.75 | 25 | 2000 | - | 1.21 |
| 0.75 | 30 | 3000 | 835 | 1.5 |
| 0.75 | 35 | 4000 | 1010 | 2 |
| 0.76 | 20 | 1000 | - | 1.8 |
| 0.76 | 25 | 2000 | 435 | 2.2 |
| 0.76 | 30 | 3000 | 620 | 0.8 |
| 0.76 | 35 | 4000 | 1411 | 1.4 |
| 0.77 | 20 | 1000 | 1008 | 2.4 |
| 0.77 | 25 | 2000 | 800 | 3 |
| 0.77 | 30 | 3000 | 422 | 1.9 |
| 0.77 | 35 | 4000 | 1530 | 2.1 |
| 0.78 | 20 | 1000 | - | 2.35 |
| 0.78 | 25 | 2000 | - | 3.11 |
| 0.78 | 30 | 3000 | 1212 | 3.22 |
| 0.78 | 35 | 4000 | 1452 | 4.1 |

NB* samples were stored at different conditions as specified to see effect on $AfB_1$ and DON production.

**Table 4.35: Effect of *X. aethiopica* spice powder, $a_w$ and temperature on the production of $AfB_1$ and DON**

| $a_w$ (%) | Temp. (°C) | *Xylopia aethiopica* (ppm) | $AfB_1$ (pg/g) | DON (ng/g) |
|---|---|---|---|---|
| 0.75 | 20 | 1000 | - | - |
| 0.75 | 25 | 2000 | 1050 | - |
| 0.75 | 30 | 3000 | 1220 | 3.1 |
| 0.75 | 35 | 4000 | 1510 | 4.2 |
| 0.76 | 20 | 1000 | 1072 | 4.15 |
| 0.76 | 25 | 2000 | 1120 | 5.26 |
| 0.76 | 30 | 3000 | 1305 | 6.5 |
| 0.76 | 35 | 4000 | 1320 | 7.2 |
| 0.77 | 20 | 1000 | 1212 | 6.05 |
| 0.77 | 25 | 2000 | 1415 | 8.1 |
| 0.77 | 30 | 3000 | 1480 | 11.2 |
| 0.77 | 35 | 4000 | 2010 | 10.5 |
| 0.78 | 20 | 1000 | - | - |
| 0.78 | 25 | 2000 | - | 4.25 |
| 0.78 | 30 | 3000 | 1005 | 5.77 |
| 0.78 | 35 | 4000 | 1115 | 6.08 |

NB* samples were stored at different conditions as specified to see effect on $AfB_1$ and DON production.

temperature. DON was not produced at water activity of 0.75, temperature of 20 $^{o}$C and *C. longa* spice addition of 1000 µg/g. All other given conditions favour the production of DON though minimally. Production of deoxynivalenol increased with increasing temperature and thus reduced the impact of *C. longa* addition on the medium and to ensure the sustenance of the impact of this spice, temperature must be maintained at 20 $^{o}$C. This showed consistency against DON producing mold and may replace chemical method like ozone which lacks consistency in effectiveness (Luo *et al.*, 2018). It is recommended that production of deoxynivalenol toxin can be minimized at water activity of 0.75 or 0.76, temperature of 20 $^{o}$C and *C. longa* spice addition level of 1000- 2000 µg/g.

Table 4.35 shows the interaction between available water, temperature and crude powder of *Xylopia aethiopica* spice on production of $AfB_1$ and DON. At constant available water ($a_w$) and varied temperature, production of aflatoxin $B_1$ increased significantly ($p< 0.01$) not minding corresponding increase in added *Xylopia aethiopica* spice. Hassane *et al*. (2017) had reported maximum growth of *Aspergillus flavus* at 33-35 $^{o}$C and a decrease as storage temperature was reduced. The use of *Xylopia aethiopica* spice in this study, to form synergy of barriers with other environmental factors, mainly temperature and available water; against production of $AfB_1$ helps confer effectiveness. Thus, quantified values of aflatoxin $B_1$ in tea in the presence of this synergy, and at every combination was less than 20 µg/kg stipulated in the regulatory standard for $AfB_1$ in foods meant for direct consumption by man. In the same vein, Table 4.35 shows the impact of crude extract of *Xylopia aethiopica* spice in combination with available water and temperature on production of deoxynivalenol toxin. Available water, $a_w$, of 0.75 and reduced temperature of 20 and 25 $^{o}$C extricate tea of deoxynivalenol toxin. At water activity level of 0.78 and temperature of 20 $^{o}$C, deoxynivalenol toxin was not found in the medium. This result is no less in agreement with Lappa *et al*. (2017) and Islam *et al.* (2013) on the required optimum condition for growth of microbes and production of fungal toxins. Therefore, holding temperature plays a very important role in forming a synergy with added spice while mitigating mycotoxins production. Table 4.36 revealed the effectiveness of interaction between crude extract of *Piper guineense*, available water, $a_w$, and temperature on production of $AfB_1$ and DON in tea. $AfB_1$ was not detected at available water of 0.75,

**Table 4.36: Effect of *P. guineense* spice powder, $a_w$ and temperature on the production of $AfB_1$ and DON**

| $a_w$ (%) | Temp. (°C) | *Piper guineense* (ppm) | $AfB_1$ (pg/g) | DON (ng/g) |
|---|---|---|---|---|
| 0.75 | 20 | 1000 | - | - |
| 0.75 | 25 | 2000 | 725 | - |
| 0.75 | 30 | 3000 | 900 | 8.25 |
| 0.75 | 35 | 4000 | 1030 | 6.33 |
| 0.76 | 20 | 1000 | - | 15.2 |
| 0.76 | 25 | 2000 | 550 | 13.2 |
| 0.76 | 30 | 3000 | 610 | 13.5 |
| 0.76 | 35 | 4000 | 1218 | 11.1 |
| 0.77 | 20 | 1000 | 700 | 14.1 |
| 0.77 | 25 | 2000 | 810 | 12.5 |
| 0.77 | 30 | 3000 | 615 | 10.2 |
| 0.77 | 35 | 4000 | 1005 | 6.41 |
| 0.78 | 20 | 1000 | - | 9.51 |
| 0.78 | 25 | 2000 | 910 | 6.11 |
| 0.78 | 30 | 3000 | 1185 | 5.1 |
| 0.78 | 35 | 4000 | 1360 | 3.1 |

NB* samples were stored at different conditions as specified to see effect on $AfB_1$ and DON production.

**Table 4.37: Summary of regression analysis of the model**

| Spice | Toxins | Mean | P-value | $R^2$ |
|---|---|---|---|---|
| *C. longa* | $AfB_1$ (pg/g) | 690.94 ± 0.1 | 0.005 | 0.851 |
| | DON (ng/g) | 2.138 ± 0.08 | 0.011 | 0.742 |
| *X. aethiopica* | $AfB_1$ (pg/g) | 1036.34±0.12 | 0.004 | 0.908 |
| | DON (ng/g) | 5.116 ± 0.05 | 0.016 | 0.785 |
| *P.guineenses* | $AfB_1$ (pg/g) | 725.63 ± 0.11 | 0.006 | 0.8 |
| | DON (ng/g) | 8.596 ± 0.04 | 0.05 | 0.751 |

temperature 20 $^{o}$C and added spice concentration of 1000 µg/g. Inspite of increase in concentration of the spice, $AfB_1$ quantified also increased suggesting that holding temperature is vital to toxins production thus agreeing with Hassane *et al*. (2017). Similarly, Table 4.36 showed a different trend with DON. Though at 0.75, aw and at a temperature of 20 $^{o}$C and 25 $^{o}$C respectively, no deoxynivalenol toxin was found, still at temperature of 30 and 35 $^{o}$C, a reduction in DON toxins was observed. The reducing trend of DON in this condition was similar. Thus, synergistic effect of $a_w$, temperature and concentration of *P. guineense* reduced DON.

In all, the parameters combined and monitored for optimum performance of the spices showed an agreement with the claims of Thanushree *et al.* (2019). Conditions that promote fungal growth may not always lead to mycotoxin production. However, generally, a temperature range between 25 and 30 °C, available water ($a_w$) higher than 0.78, and relative humidity between 88% and 95% are considered as favorable for fungal growth and subsequent mycotoxin production.

Table 4.37 presents the summary of predictive models for $AfB_1$ and DON production at different available water, temperature and spice addition (Cl, Xa, Pg). The mean value for the produced $AfB_1$ and DON under the influence of *C. longa* spice, were 690.94 pg/g and 2.138 ng/g respectively. This indicates that the results is significant in the multiple linear regression model thus showing the acceptance of established model. The p-value obtained also confirms the applicability of this result since it is above 95 percent confidence level. Also, from the regression analysis, coefficient of determination, $R^2$, provided correlation between measured response (obtained from experimental run) and predicted response (obtained from multiple linear regression model). The $R^2$ value of 85 percent for $AfB_1$ agrees with the position of Al-Hassani *et al*., 2014 who submitted that the closer the $R^2$ to 100 percent, the suitable the model. This position is similar with DON production as $R^2$ of 74 percent agreed with Solaiman *et al*., (2016) which revealed that experimental data could be well explained when $R^2$ of the regression model is close to 100 %. Table 4.37 again showed the mean value of $AfB_1$ and DON production under the influence of *Xylopia aethiopica* spice in combination with available water and temperature. The mean value of 1036.34 pg/g and 5.116 ng/g obtained respectively for $AfB_1$ and DON were

significant. P-values obtained were also significant and $R^2$ values too in agreement with Al-Hassani *et al*. (2014) and Aroyeun *et al*. (2011), who used *Aframomum danielli* spice for reduction of OTA. The $R^2$ value of above 90 % for the $AfB_1$in the model, a value closer to 100%, agreed with Solaiman *et al*. (2016). Table 4.37 showed similar result of the effect of *piper guineense* spice on AfB1 and DON production with mean values of 725.63 pg/g and 8.596 ng/g respectively. An $R^2$ of 80 percent was recorded in the regression models for $AfB_1$ and 75 percent for DON. P-values obtained were also significant in both treatments.

### 4.7.3 Optimal Response of $AfB_1$ and Don to Production conditions

#### 4.7.3.1 $AfB_1$ Production at Available Water, Temperature and *C. longa*

Figure 4.3 shows the optimization plot and the optimal solution for the desired responses ($AfB_1$ production). Considering the analysis, it was predicted that the minimum $AfB_1$ produced was 0. These desired responses were achieved at 0.76 available water, 20 $^oC$, temperature and *C. longa* of 4000 ppm, and composite desirability (D) of 1.000 that was greater than 0.90 and exactly 1.00. The composite desirability (D) is also a statistical parameter to validate accuracy of the optimization plot (Moosavi and Ghassabian, 2018). It has been reported by Chang *et al*. (2015), that the optimization of factors and responses obtained from the statistical analysis is highly reliable and accurate when the composite desirability (D) is closer to 1.00. Hence, the optimal conditions proposed in the optimization plot (Figure 4.3) was generally reliable, and it fully conform with multiple linear regression model developed.

#### 4.7.3.2 DON Production at Available Water, Temperature and *C. longa*

Figure 4.4 shows the optimization plot and the optimal solution for the desired responses (DON production). Considering the analysis, it was predicted that the minimum DON produced was 0. These desired responses were achieved at 0.75 available water, 20 $^oC$ temperature and *C. longa* of 2000 ppm, and composite desirability (D) of 1.000 that was greater than 0.90 and exactly 1.00. The composite desirability (D) is also a statistical parameter to validate accuracy of the optimization plot (Moosavi and Ghassabian, 2018). It has been reported by Chang *et al*. (2015), that the optimization of factors and

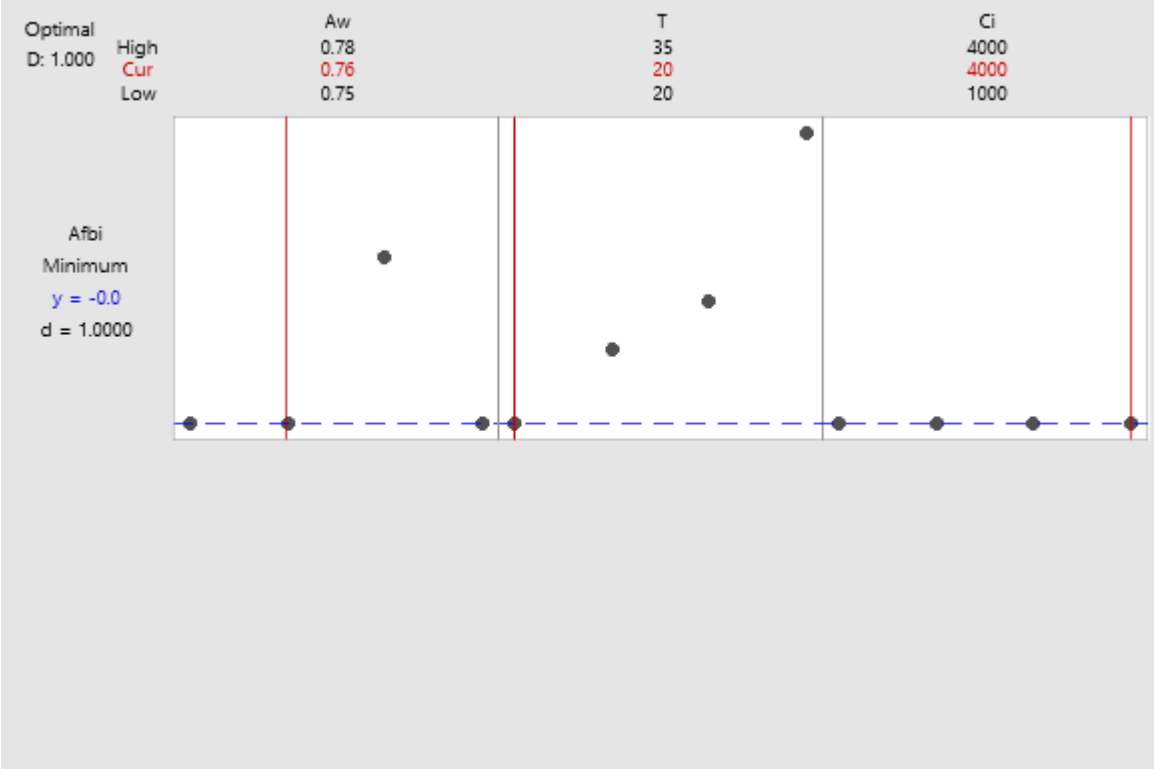


**Figure 4.3: Optimization Plot for $AfB_1$ Production (aw/T/Cl)**

responses obtained from the statistical analysis is highly reliable and accurate when the composite desirability (D) is closer to 1.00. Hence, the optimal conditions proposed in the optimization plot (Figure 4.4) was generally reliable, and it fully conforms with multiple linear regression model developed.

#### 4.7.3.3 $AfB_1$ Production at Available Water, Temperature and *X. aethiopica* (Xa) spice

Figure 4.5 shows the optimization plot and the optimal solution for the desired responses ($AfB_1$ production). Considering the analysis, it was predicted that the minimum AfB1 produced was 0. These desired responses were achieved at 0.75 available water, 20 $^oC$, temperature and *X. aethiopica* of 1000 ppm, and composite desirability (D) of 1.000 that was greater than 0.90 and exactly 1.00. The composite desirability (D) is also a statistical parameter to validate accuracy of the optimization plot (Moosavi and Ghassabian, 2018). It has been reported by Chang *et al.* (2015), that the optimization of factors and responses obtained from the statistical analysis is highly reliable and accurate when the composite desirability (D) is closer to 1.00. Hence, the optimal conditions proposed in the optimization plot (Figure 4.5) was generally reliable, and it fully conform with multiple linear regression model developed.

#### 4.7.3.4 DON Production at Available Water, Temperature and *X. aethiopica* (Xa) spice

Figure 4.6 shows the optimization plot and the optimal solution for the desired responses (DON production). Considering the analysis, it was predicted that the minimum Don produced was 0. These desired responses were achieved at 0.75 available water, 25 $^oC$, temperature and *X. aethiopica* of 1000 ppm, and composite desirability (D) of 1.000 that was greater than 0.90 and exactly 1.00. The composite desirability (D) is also a statistical parameter to validate accuracy of the optimization plot (Moosavi and Ghassabian, 2018). It has been reported by Chang *et al*. (2015), that the optimization of factors and responses obtained from the statistical analysis is highly reliable and accurate when the composite desirability (D) is closer to 1.00. Hence, the optimal conditions proposed in the

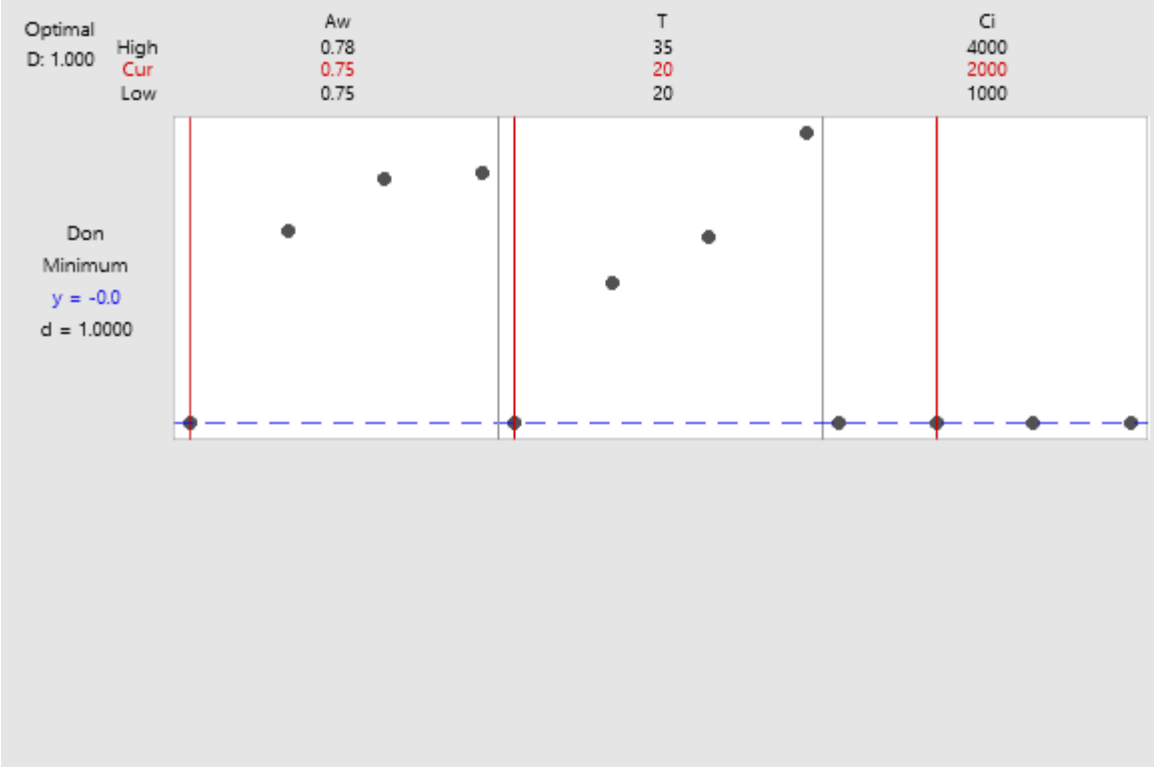


**Figure 4.4: Optimization Plot for Don Production (aw/T/Cl)**

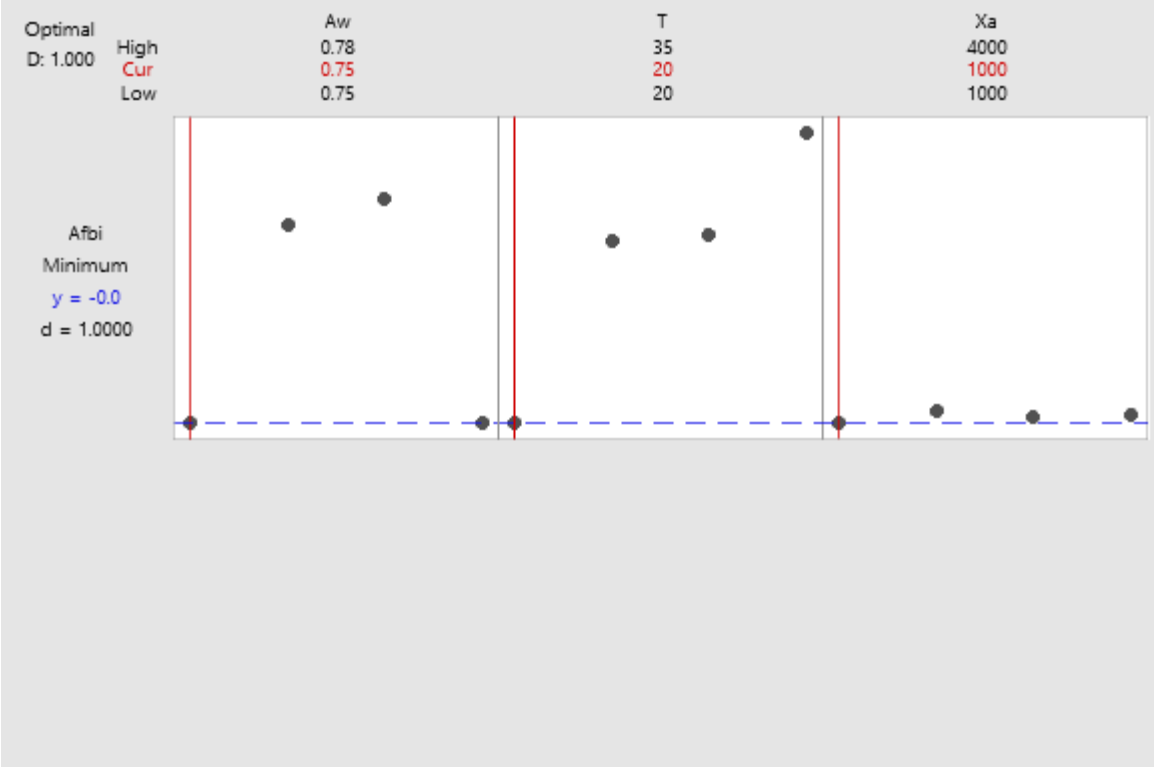


**Figure 4.5: Optimization Plot for $AfB_1$ Production (aw/T/Xa)**

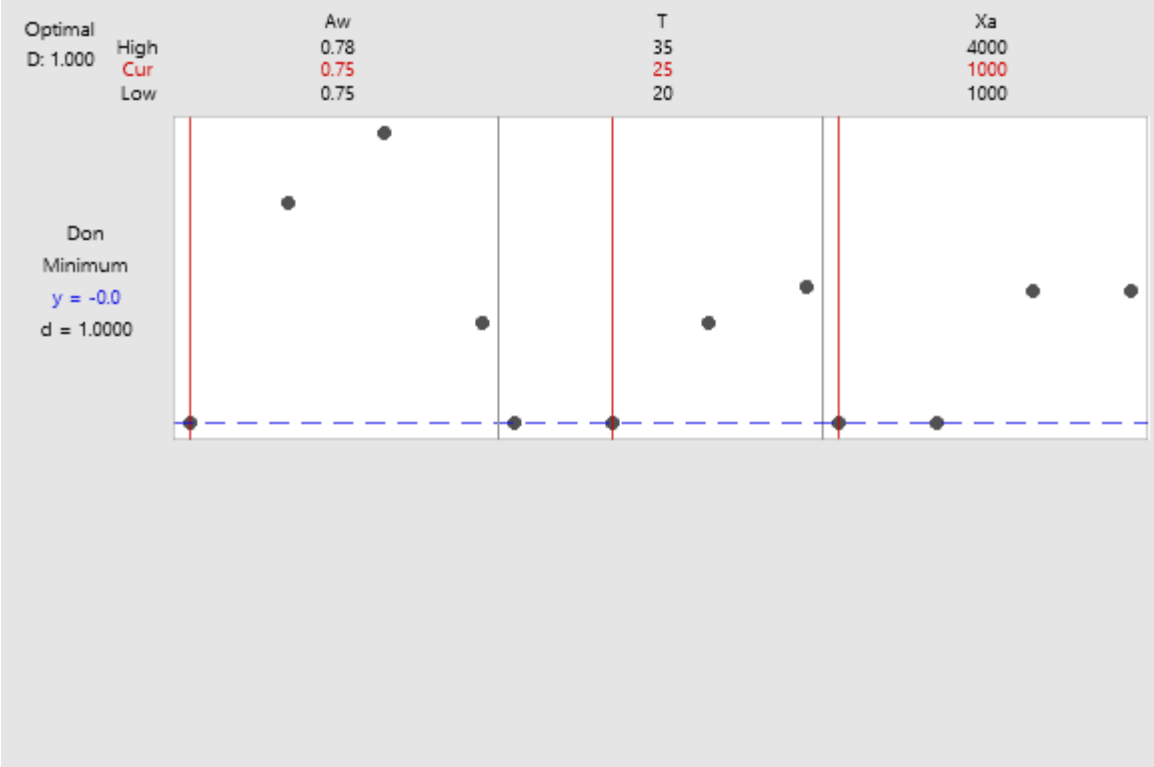


**Figure 4.6: Optimization Plot for Don Production (aw/T/Xa)**

optimization plot (Figure 4.6) was generally reliable, and it fully conforms with the multiple linear regression model developed.

#### 4.7.3.5 $AfB_1$ Production at Available Water, Temperature and *P. guineenses*

Figure 4.7 shows the optimization plot and the optimal solution for the desired responses ($AfB_1$ production). Considering the analysis, it was predicted that the minimum $AfB_1$ produced was 0. These desired responses were achieved at 0.75 available water, 20 $^oC$, temperature and *P. guineenses* of 2000 ppm, and composite desirability (D) of 1.000 that was greater than 0.90 and exactly 1.00. The composite desirability (D) is also a statistical parameter to validate accuracy of the optimization plot (Moosavi and Ghassabian, 2018). It has been reported by Chang *et al*. (2015), that the optimization of factors and responses obtained from the statistical analysis is highly reliable and accurate when the composite desirability (D) is closer to 1.00. Hence, the optimal conditions proposed in the optimization plot (Figure 4.7) was generally reliable, and it fully conform with multiple linear regression model developed.

#### 4.7.3.6 DON Production at Available Water, Temperature and *P. guineense* spice

Figure 4.8 shows the optimization plot and the optimal solution for the desired responses (DON production). Considering the analysis, it was predicted that the minimum DON produced was 0. These desired responses were achieved at 0.75 available water, 20 $^oC$, temperature and *P. guineense* of 3000 ppm, and composite desirability (D) of 1.000 that was greater than 0.90 and exactly 1.00. The composite desirability (D) is also a statistical parameter to validate accuracy of the optimization plot (Moosavi and Ghassabian, 2018). It has been reported by Chang *et al*. (2015), that the optimization of factors and responses obtained from the statistical analysis is highly reliable and accurate when the composite desirability (D) is closer to 1.00. Hence, the optimal conditions proposed in the optimization plot (Figure 4.8) was generally reliable, and it fully conform with the multiple linear regression model developed.

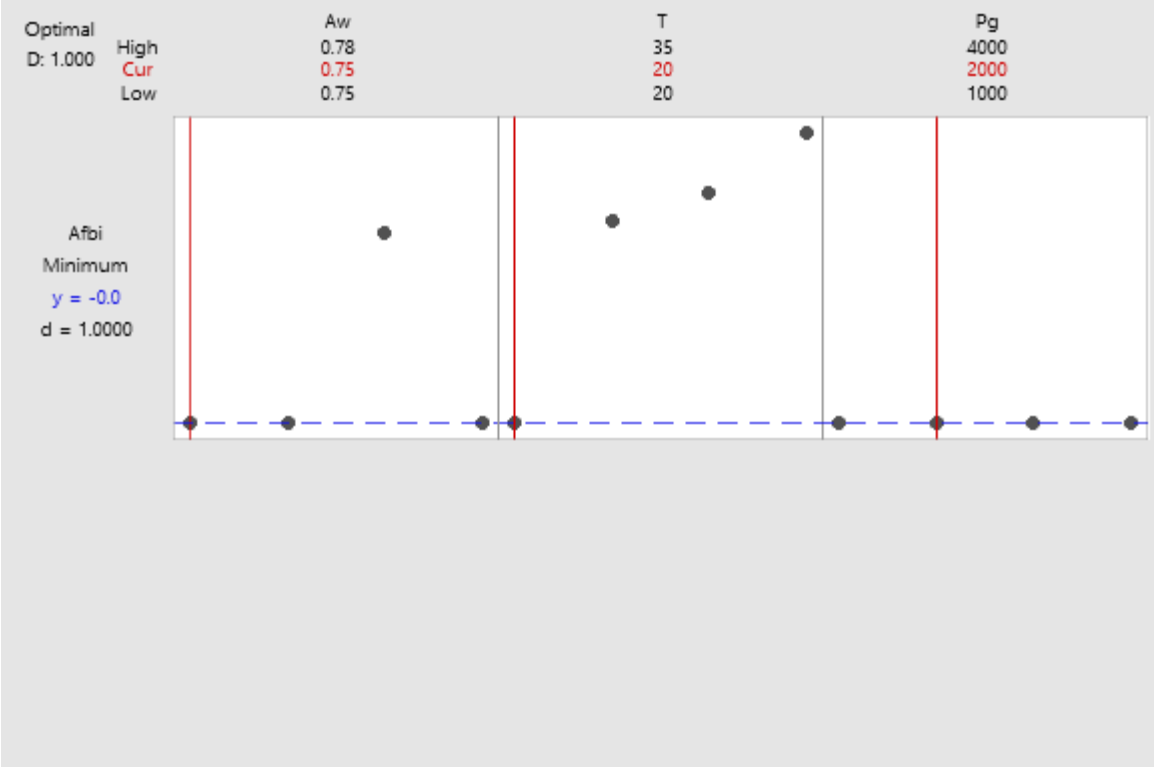


**Figure 4.7: Optimization Plot for $AfB_1$ Production (aw/T/pg)**

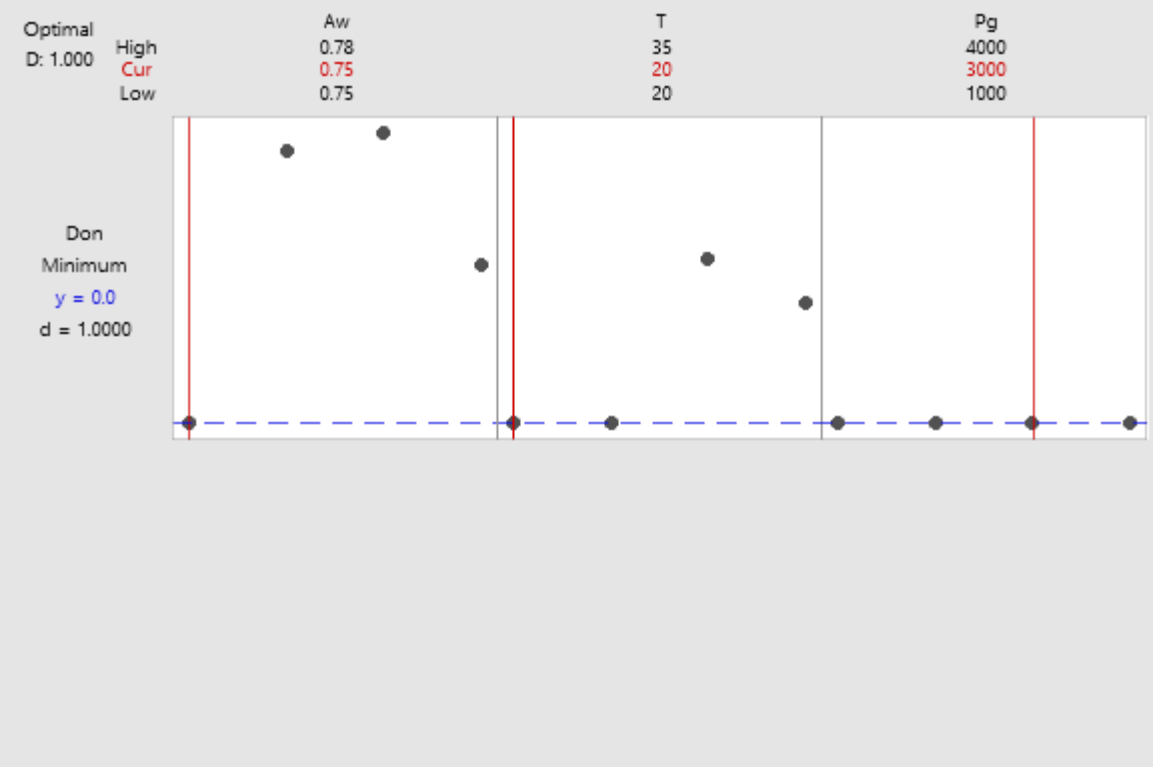


**Figure 4.8: Optimization Plot for Don Production (aw/T/pg)**

## 4.8 Phytoactive compounds in *Curcuma longa, Xylopia aethiopica* and *Piper guineense* spice

### 4.8.1 FT- IR on phyto activity of *C. longa, X. aethiopica* and *P. guineense* spices

The FT-IR of the spices was shown in Figs 4.9- 4.11**.** This was necessary to determine the functional groups present in the selected spices. Golubstova (2017), reported that the frequency range between 3800- 2600 $cm^{-1}$ mostly contained valence fluctuation frequencies of OH groups, CH2 and CH3 groups. Also, frequencies between 1800- 1200 $cm^{-1}$ indicated the characteristic frequencies of the valence vibrations of C=O and –C=O deformation vibrations of methylene and methyl groups as well as –OH groups. Infrared of *Curcuma longa,* (Table 4.38), *Xylopia aethiopica,* (Table 4.39) and *Piper guineense,* (Table 4.40), showed common stretching vibration at 3200 – 3550 $cm^{-1}$ due to O-H groups, C-O aromatic stretching vibration at 1200 – 1275 $cm^{-1}$ of an alkyl aryl ether, N-O stretching of nitro compound at 1550 – 1500 $cm^{-1}$ and high intensity band at 1512 $cm^{-1}$attributed to the mixed vibrations including stretching carbonyl bond vibrations; C=C stretching of conjugated alkenes at 1627 $cm^{-1}$. The band at 2925.64 $cm^{-1}$ was due to C-H3, C-H2 and C-H. S=O of sulfoxide and C-O of tertiary alcohol stretching are common to both *Xylopia aethiopica* and *Piper guineense* at 1051 and 1159 $cm^{-1}$. Other distinctive bands perculiar to *Xylopia aethiopica* include S=O stretching of sulfone and sulphate at 1320 $cm^{-1}$ and 1380 $cm^{-1}$ respectively, C-H bending of 1,2,3 tri-substituted chain at 778 $cm^{-1}$ and at the presence of benzene derivative at 720 $cm^{-1}$.

The results of the present study further revealed that the FT-IR analysis on the powder extracts of *Curcuma longa*, *Xylopia aethiopica* and *Piper guineense* separated the functional groups of the component, based on its peak ratio which identified the chemical compounds. The presence of alchohols, phenols, carboxylic acids, ethers, aromatics, aryl ketone, alkenes and saturated aldehyde might be responsible for various antioxidant properties of the spices with *Xylopia aethiopica* having possibility of greater antioxidant properties because of tri-substitution multiple sulphur compounds available in its molecule.

**Table 4.38 Functional groups in *Curcuma longa* spice**

| Absorption | Appearance | Group | Compound/Class |
|---|---|---|---|
| 3550- 3200 (3411) | Strong, broad | O-H stretching | Alkane |
| 3000- 2840 (2924) | Medium | C-H stretching | Alkane |
| 3000- 2840 (2852) | Medium | C-H stretching | Alkane |
| (2354) | - | - | |
| (2307) | - | - | |
| 1650- 1600 (1627) | Medium | C=C stretching | Conjugated alkene |
| (1602) | Medium | C=C stretching | Conjugated alkene |
| !550- 1500 (1511) | Strong | N-O stretching | Nitro compound |
| 1440- 1395 (1430) | Medium | O-H bending | Carboxylic acid |
| 1390- 1380 (1378) | Medium | C-H bending | Aldehyde |
| 1310- 1250 (1280) | Strong | C-O stretching | Aromatic ester |
| (1207) | Strong | C-O stretching | Akyl amyl ether |
| 1250- 1020 (1028) | Medium | C-N stretching | Amine |
| 850-550 (766) | Strong | C-Cl stretching | Halo compound |
| 730- 665 (706) | Strong | C=C bending | Alkene |
| 690- 515 (576) | Strong | C-Br stretching | Halo compound |

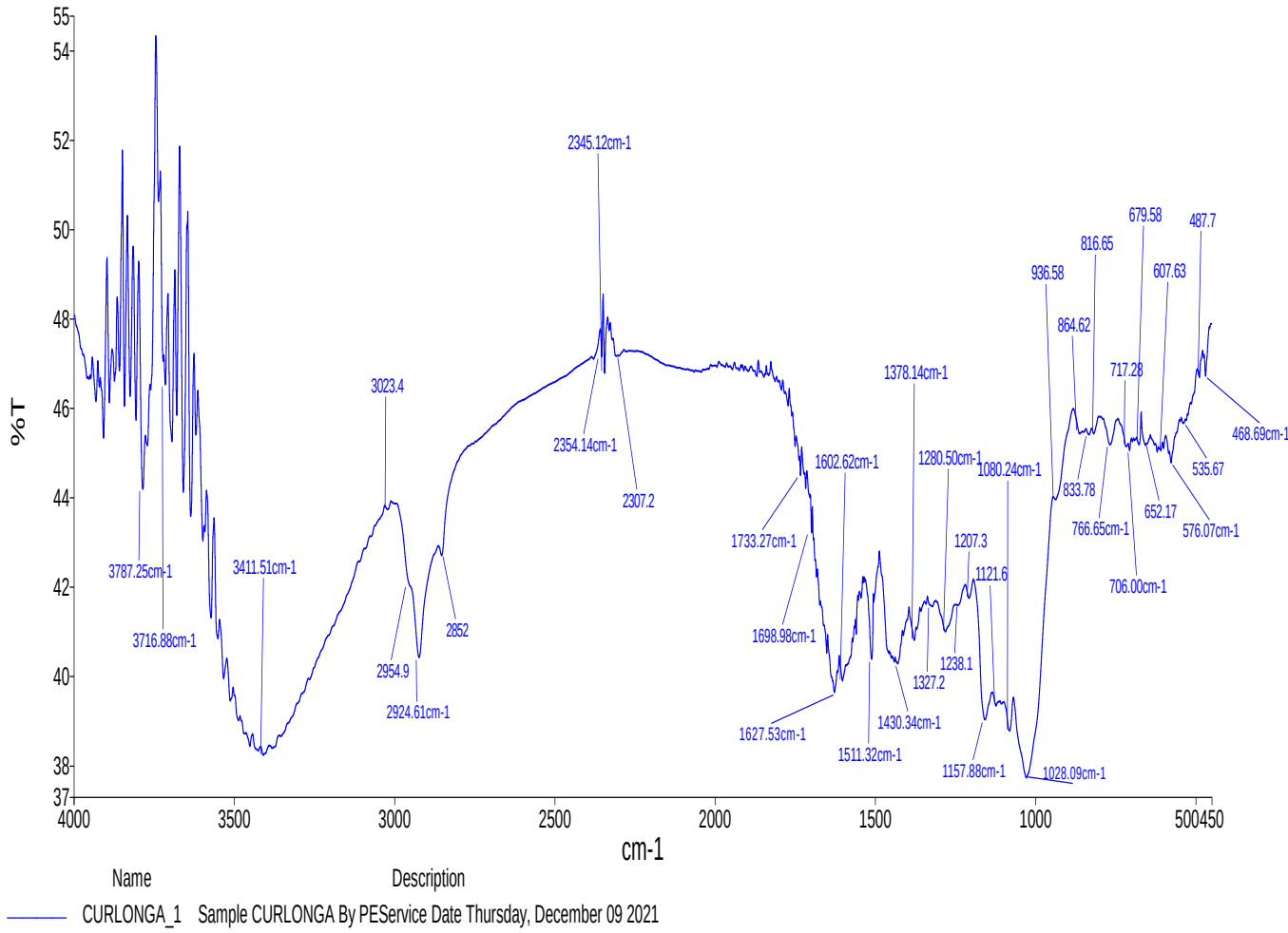


**Figure 4.9: FTIR spectra for *Curcuma longa* spice**

**Table 4.39 Functional groups in *Xylopia aethiopica* spice**

| Absorption | Appearance | Group | Compound/Class |
|---|---|---|---|
| 3550- 3200 (3433) | Strong, broad | O-H stretching | Alcohol |
| 3000- 2840 (2924) | Medium | C-H stretching | Alkane |
| 2400- 2000 (2345) | Strong | O=C=O stretching | |
| 1650- 1600 (1628) | Medium | C=C stretching | Conjugated alkene |
| 1550- 1500 (1522) | Strong | N-O stretching | Nitro compound |
| 1500- 1450 (1454) | Medium | C-H bonding | Alkane |
| 1415- 1380 (1382) | Strong | S=O stretching | Sulfate |
| 1350- 1300 (1320) | Strong | S=O stretching | Sulfone |
| 1275- 1200 (1251) | Strong | C=O stretching | Akyl amyl ether |
| 1205- 1124 (1159) | Strong | C-O stretching | Tertiary alcohol |
| 1070- 1030 (1051) | Strong | S=O stretching | Sulfoxide |
| 895- 885 (895) | Strong | C=C bending | Alkene |
| 840- 790 (826) | Medium | C=C bending | Alkene |
| 780- 720 (778) | Strong | C-H bending | 1,2,3 trisubderivatives |

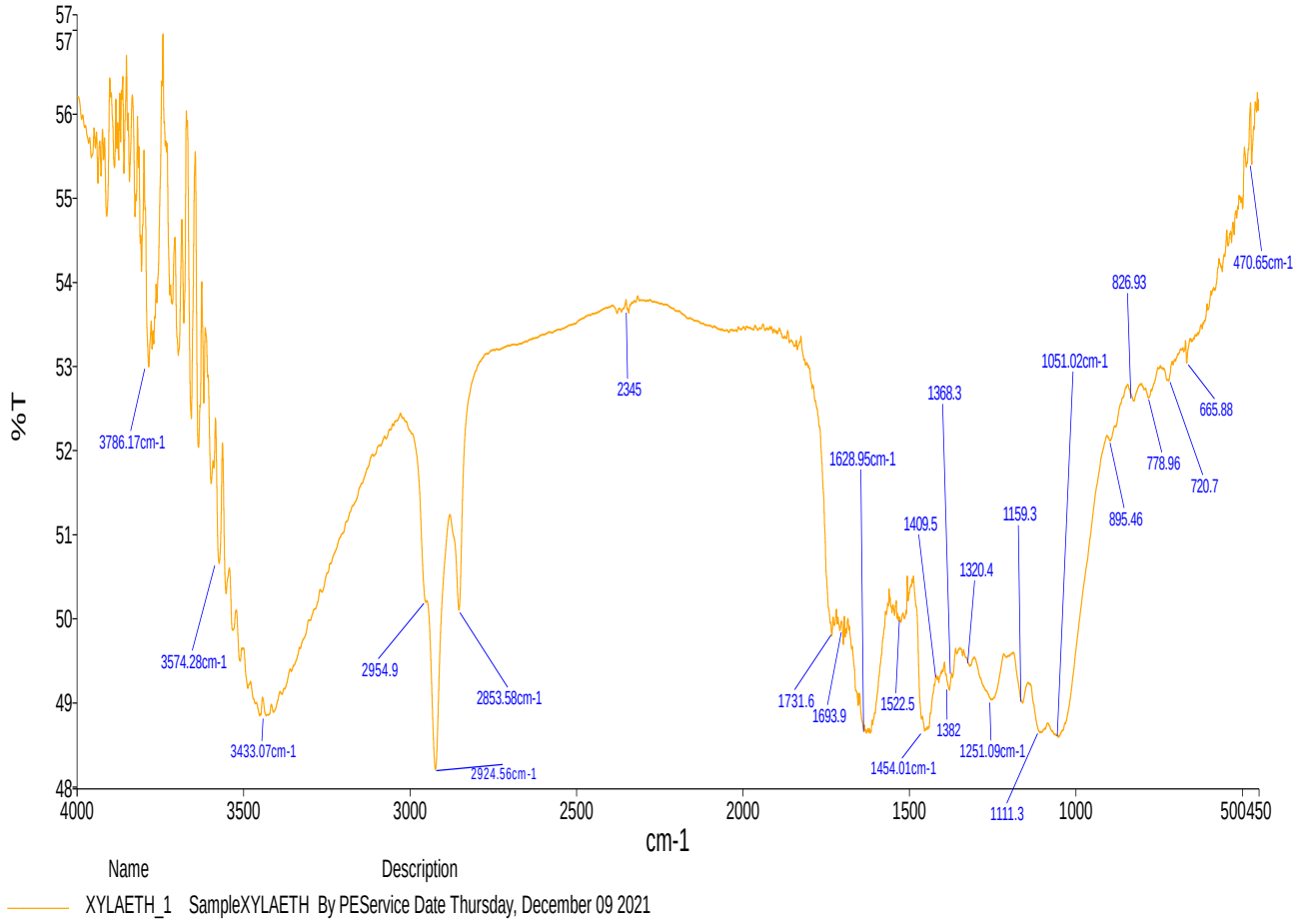


**Figure 4.10: FTIR spectra for *Xylopia aethiopica* spice**

**Table 4.40 Functional groups in *Piper guineense* spice**

| Absorption | Appearance | Group | Compound/Class |
|---|---|---|---|
| 3550- 3200 (3419) | Strong, broad | O-H stretching | Alcohol |
| 3000- 2840 (2925) | Medium | C-H stretching | Alkane |
| 2840- 2349 (2341) | Strong | O=C=O stretching | $CO_2$ |
| 2000- 1650 (1638) | Weak | C=H bending | Aromatic compound |
| 1650- 1450 (1443) | Medium | C-H bending | Alkane |
| 1550- 1500 (1503) | Strong | N-O stretching | Nitro compound |
| 1275- 1200 (1251) | Strong | C-O stretching | Akyl amyl ether |
| 1205- 1124 (1155) | Strong | C-O stretching | Tertiary alcohol |
| 1070- 1030 (1039) | Strong | S=O stretching | Sulfoxide |

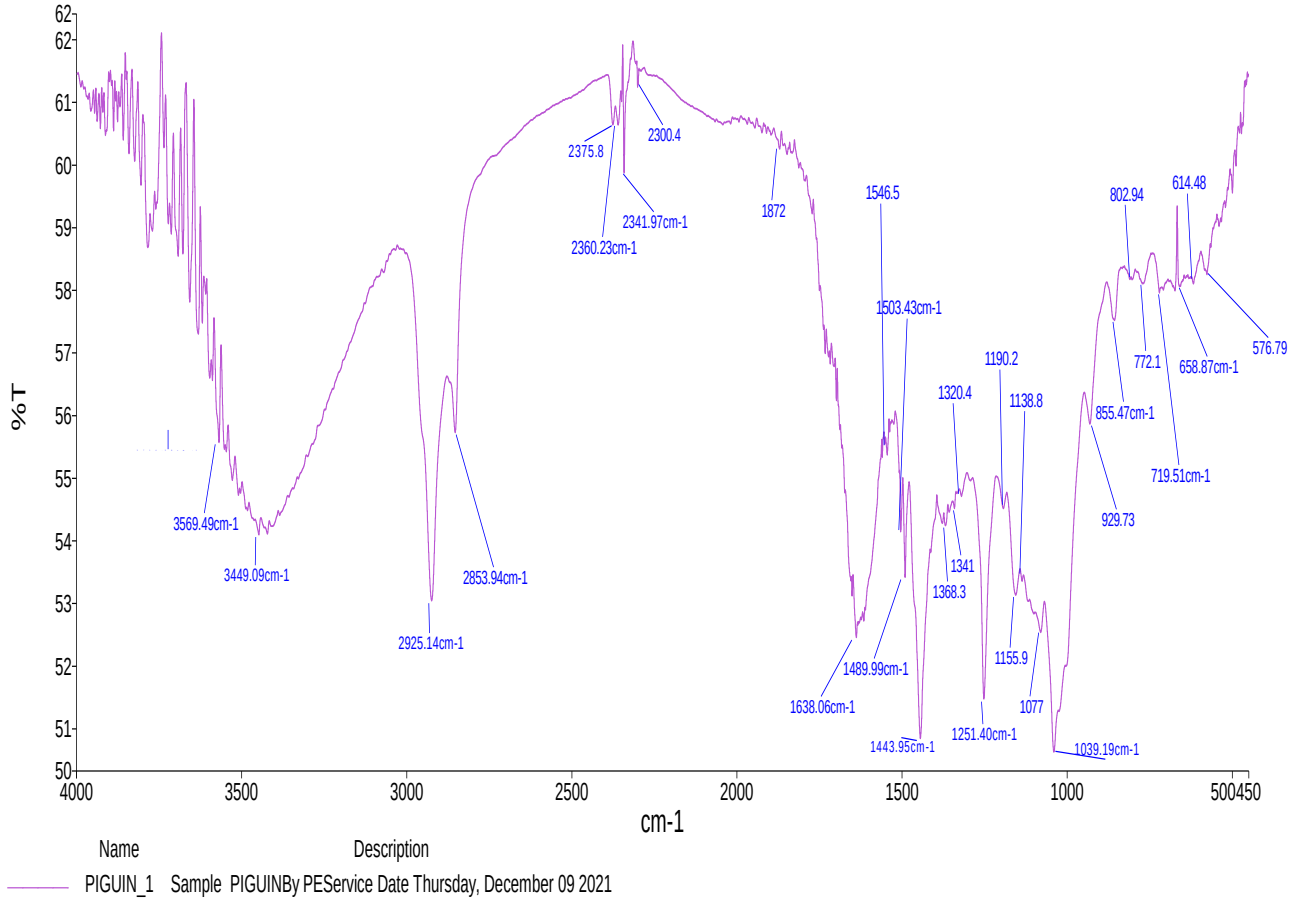


**Figure 4.11: FTIR spectra for *Piper guineense* spice**

### 4.9.0 Sensory evaluation of tea-spice blends

Mean values of the multiple comparison analysis for attributes of colour, appearance, smell/aroma, taste and overall acceptability of tea treated with crude powder extract of *Curcuma longa*, *Xylopia aethiopica* and *Piper guinenses* were shown in Table 4.41.

**Colour:** the colour attributes of tea beverage indicated significant differences ($p< 0.05$) between sample that lack spice (control) and other samples. Beverages represented with sample codes MC4B.3000Cl, MC4B.4000Cl, MC4B.1000Xa, MC4B.3000Xa, MC4B.4000Xa, MC4B.1000Pg, MC4B.3000Pg and MC4B.4000Pg were not significantly different in terms of colour except for sample MC4B.1000Cl. Sample coded MC4B.4000Pg ranked least for all the tea samples. Control (No spice) and sample MC4B.1000Cl (1000ppm added *C. longa*) compared favourably as there was no significant difference ($p < 0.05$) between them. Reduction in brilliancy of colour with increased addition of spice powder from *X. aethiopica*, and *P. guinense* also confer reduction in acceptability; as shown by mean values, of the tea-spice blend beverage.

**Appearance:** there is no significant difference ($p< 0.05$) between control (no spice) and sample MC4B.1000Cl (1000ppm). Also, difference does not exist between samples coded MC4B.1000Xa and MC4B.1000Pg, but significant difference exists as spice addition increased.

**Taste**: the preferred taste of the control sample (no spice) is not unexpected as infused tea containing spice blend as treatment, seem to be a new product to the panelists due to unfamiliar taste perception, hence a different rating response. The addition of spice powder extracts namely; *Curcuma longa*, *Xylopia aethiopica* and *Piper guinense*, to tea beverage impart a new perception (Le and Worch, 2018). As much as the tea beverage treated with spices falls in the 'like category', it is convenient to deduce that attributes of the selected spices have been transferred to the tea beverage thus complements the characteristics of tea. The inclusion of spice powder at varying concentrations (1000ppm, 3000ppm, and 4000ppm), created a new product with characteristic acceptable perception in taste as one of the strongest predicators of food choices. The result obtained agreed with that of Kollar-Hunek and Herbeger (2013), who also submitted that tasters evaluate food based on personal values of which good taste was very important. Good taste is linked to the perceived quality of food (Sipos *et al*., 2017). Therefore, taste decreased in

**Table 4.43: Sensory evaluation of infused tea treated with different concentration of tropical spices**

| Sample code | Colour | Appearance | Smell/Aroma | Taste | Overall acceptability |
|---|---|---|---|---|---|
| No spice | 7.58±0.48$^{a}$ | 7.73±0.50$^{a}$ | 7.69±0.43$^{a}$ | 7.54±0.47$^{a,b}$ | 7.85±0.33$^{a,b}$ |
| MC**4**B.1000Cl | 7.31±0.50$^{b}$ | 7.27±0.53$^{a,b}$ | 7.58±0.47$^{a}$ | 7.77±0.45$^{a}$ | 7.65±0.34$^{b,c}$ |
| MC**4**B.3000Cl | 6.15±0.56$^{e,f}$ | 6.54±0.59$^{c,d}$ | 5.73±0.61$^{d}$ | 6.12±0.53$^{e}$ | 7.50±0.35$^{c}$ |
| MC**4**B.4000Cl | 7.04±0.53$^{b,c,d}$ | 7.04±0.56$^{b,c}$ | 7.54±0.48$^{a}$ | 7.08±0.50$^{b,c,d}$ | 8.00±0.31$^{a}$ |
| MC**4**B.1000Xa | 6.77±0.54$^{c,d}$ | 6.96±0.57$^{b,c}$ | 6.85±0.49$^{b}$ | 6.92±0.51$^{c,d}$ | 7.00±0.36$^{d}$ |
| MC**4**B.3000Xa | 5.89±0.76$^{f}$ | 6.31±0.60$^{d,e}$ | 6.27±0.50$^{c}$ | 6.65±0.52$^{d}$ | 6.31±0.37$^{f,g}$ |
| MC**4**B.4000Xa | 7.12±0.52$^{b,c}$ | 7.15±0.55$^{b}$ | 7.62±0.45$^{a}$ | 7.23±0.49$^{b,c}$ | 7.46±0.35$^{c}$ |
| MC**4**B.1000Pg | 6.69±0.55$^{c,d}$ | 6.85±0.58$^{b,c,d}$ | 6.39±0.50$^{c}$ | 6.65±0.52$^{d}$ | 6.85±0.36$^{d,e}$ |
| MC**4**B.3000Pg | 5.92±0.57$^{f}$ | 5.92±0.85$^{e}$ | 5.96±0.51$^{c,d}$ | 6.12±0.53$^{e}$ | 6.12±0.32$^{g}$ |
| MC**4**B.4000Pg | 6.54±0.56$^{d,e}$ | 6.54±0.59$^{c,d}$ | 6.39±0.49$^{c}$ | 6.04±0.68$^{e}$ | 6.54±0.36$^{e,f}$ |

a,b,c…. means along same vertical column with same superscript are not significantly different at $p<0.05$.

the order as observed on Table 4.41 and still with exception as MC4B.1000Cl > No spice. Tea blended with 1000ppm *C. longa* was ranked best when compared with control and tea blended with *Piper guinense* ranked least for taste attributes.

**Smell/Aroma**: No significant difference ($p < 0.05$) in aroma for *C. longa* spice added at all concentration (1000ppm, 3000ppm, 4000ppm) examined and this is also similar with *P.guinense* spice blended tea. Difference exists for *X. aethiopica* blended tea samples as panelists preferred 4000ppm addition over 1000ppm and 3000ppm inclusions respectively.

**Overall acceptability**: *Curcuma longa* treated tea was well accepted over *Xylopia aethiopica* and *Piper guinense* treated tea samples (C. longa > X. aethiopica > P. guinense). *Curcuma longa* treated tea with 4000ppm inclusion level was ranked as well as the control. No significant difference ($p < 0.05$) between control sample and the tea coded MC4B.1000Cl. *Piper guinense* treated teas were scored least in terms of acceptability.

## 4.10 Correlation of sensory attributes in spice treated tea

Table 4.42 showed that acceptability of spice treated tea beverage had a significantly strong relationship to all other sensory attributes like taste ($p < 0.05$), aroma ($p < 0.05$), appearance ($p < 0.05$) and colour. There was abundant evidence of the dominating role of sensory appeal, especially taste, in accepting or rejecting a particular food (Eertmans *et al*., 2005). Correlation was significant between colour and taste ($p < 0.05$), colour and appearance ($p < 0.05$), and colour and aroma ($p < 0.01$). Aroma also had strong correlation to taste ($p < 0.01$) and appearance ($p < 0.05$).

**Table 4.42 Correlation of sensory parameters of infused tea treated with *Curcuma longa, Xylopia aethiopica* and *Piper guineense***

| Parameters | Colour | Appearance | Aroma | Taste |
|---|---|---|---|---|
| Acceptability | 0.64422** | 0.60710** | 0.71615** | 0.69120** |
| Colour | | 0.60558** | 0.50175** | |
| Appearance | 0.60558** | | 0.31855* | |
| Aroma | 0.50175** | 0.31855* | | 0.60384** |
| Taste | | | 0.60384** | |

* Correlation is significant at $p<0.05$, ** Correlation is highly significant at $p<0.01$

# CHAPTER FIVE

# SUMMARY, CONCLUSION AND RECOMMENDATIONS

## 5.1 Summary

Tea and coffee obtained from warehouses, upon examination, showed presence of fungi and mycotoxin contamination especially $AfB_1$ and DON, which are of health concern. Tea and coffee were processed to green and black varieties by conventional methods and treated with powder spice obtained from *Curcuma longa* roots, *Xylopia aethiopica* and *Piper guineense* seeds. The treated tea and coffee as well as control were kept in tea bags and stored for a period of six months under controlled and ambient conditions. The efficacy of spice treatment was evaluated.

Total phenolic content of the spice treated tea and coffee, improved significantly. Seventy toxins were quantified in the tea and coffee. Reduction of fungal growth and mycotoxins production in the green and black tea/coffee varieties was observed at all levels of spice treatment. *Xylopia aethiopica* spice showed higher potency at reducing fungal growth and mycotoxin contamination in the tea and coffee.

## 5.2 Conclusion

Total phenolic content of green tea, black tea, green coffee and black coffee were determined and found to be present in appreciable quantities. Incorporating spices into these materials improved significantly, the polyphenolic content of the blends. Tea commodities stored under controlled atmosphere did not grow fungi, while those stored at room temperature had loads of fungi. Strains of mycotoxigenic fungi were detected in the tea and coffee commodities assessed and these micro organisms were representative of the sample source. One new strain of *Aspergillus* specie was discovered. About seventy (70) mycotoxin contaminants were quantified in tea and coffee obtained from Ibadan, Oyo

State and Mambilla in Taraba State using high performance liquid chromatography (HPLC LC/MS). These toxin contaminants were present in tea and coffee at varying concentration; some higher than specified acceptable levels and others below limits of detection. Specifically, mycotoxins contamination level in green tea and green coffee was minimal but worrisome in black tea and coffee varieties. Effectiveness of the reducing strength of selected tropical spices, namely, *Curcuma longa*, *Xylopia aethiopica* and *Piper quineense* were evaluated on mycotoxins in teas with contamination above acceptable threshold. Crude powder of *Curcuma longa*, *Xylopia aethiopica* and *Piper guineense* spices were effective in reducing $AfB_1$ and DON contaminants in tea. *Curcuma longa* spice reduced $AfB_1$ significantly below the EU regulatory limit of 2-8µg/kg specified for food items intended for direct consumption. It also reduced DON appreciably by about fifty percent. *Xylopia aethiopica* spice has higher reducing power than *Curcuma longa* and *Piper guineense* spices. While the reducing efficiency of *Curcuma longa* and *Piper guineense* were dependent on increased quantities of the spices, efficacy of *Xylopia aethiopica* increased at smaller quantity. There is no difference in appearance, smell/aroma and taste of infused tea treated with 1,000 µg/g powder from *Curcuma longa* as well as control whereas, difference ($p< 0.05$) exists in colour. Meanwhile, significant differences exists with 1,000 µg/g of *Xylopia aethiopica*, *piper guineense* and control.

## 5.3 Recommendations

It is recommended that the new strains of Aspergillus specie discovered be named and placed in public domain (further studies). Also, tea and coffee should be dried to low moisture content of seven (7%) percent and stored in controlled atmosphere (40- 50 percent humidity) where practicable. Where controlled atmosphere storage is not practicable, blending powder of *Curcuma longa*, *Xylopia aethiopica* and *Piper guineense* with teas in combination with storage at ambient temp inhibited or stemmed the growth of fungi and production of mycotoxin, thus ensured safety, of teeming consumers of tea beverages, from exposure to hazardous mycotoxigenic substances. Sensory evaluation study carried out showed no significant difference between the spiced tea and regular tea served to the panelists, hence spiced teas is recommended. Also, good hygeine practice,

good manufacturing practice and constant training of the production operators will help to enhance delivery of quality and safe tea and coffee for consumers at all times.

Since indications regarding *Xylopia aethiopica* spice revealed that smaller concentration was required to reduce mycotoxins in tea, further studies should be done to determine exact quantity required to effect mycotoxin reduction viz-a-viz time frame of action. Again, studies is required on the array of mycotoxin contaminants found in green teas orchestrated by other fungi group as reported in this study. There is need to determine the toxic impact of these mycotoxins by conducting rat feeding experiment and thus establish minimal exposure to these toxins.

## 5.4 Contributions to knowledge

i. The study revealed processed commercial teas and coffee were contaminated with new strains of *aspergillus* species, alongside other toxigenic organisms and various mycotoxins

ii. It was established that black teas contain high quantity of aflatoxin $B_1$ ($AfB_1$) and deoxynivalenol toxins

iii. The active components (terpenes) in *Curcuma longa* spice, *Xylopia aethiopica* spice and *Piper guineense* spice reduced significantly the level of $AfB_1$ and deoxynivalenol toxins in black tea

iv. The optimum combination for each of the spices was established. *Xylopia aethiopica,* inhibited aflatoxin $B_1$ on the addition of 1000 µg/g, holding temperature of 20 $^oC$ and available water of 75 percent. *Xylopia aethiopica* also inhibited deoxynivalenol at the addition of 1000 µg/g, holding temperature of 25 $^oC$ and available water of 75 percent. *Curcuma longa* inhibits $AfB_1$ on addition of 4000 µg/g, at a holding temperature of 20 $^oC$ and available water of 76 percent. This same spice inhibited DON when 2000 µg/g was added at a holding temperature of 20 $^oC$ and available water of 75 percent. Lastly, *Piper guineense* spice inhibited $AfB_1$ production on addition of 2000 µg/g at a holding temperature of 20 $^oC$ and available water of 75 percent. It also inhibited DON production on addition of 3000 µg/g, at a holding temperature of 20 $^oC$ and available water of 75 percent.

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# APPENDICES

## Appendix $A_1$: Green Tea leaves

## $A_2$: Black Tea leaves

## $A_3$: Packaged Tea

**Appendix $B_1$: Green coffee beans**

**$B_2$: Black coffee beans**

**Appendix $C_1$: Diced and dried *Curcuma longa* rhizome**

**Appendix $C_2$: Powdered *Curcuma longa* spice**

**Appendix $D_1$: Dried pods of *Xylopia aethiopica* spice**

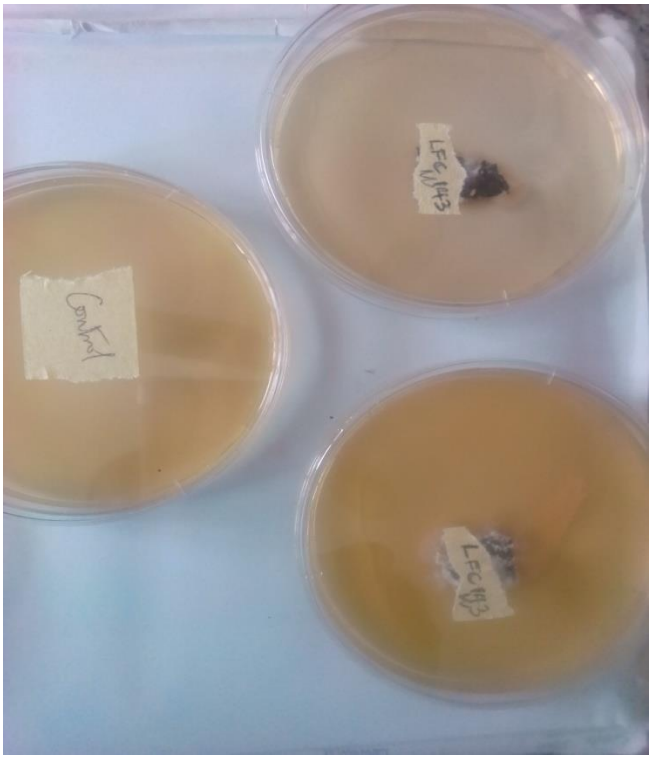
**Appendix $D_2$: *Xylopia aethiopica* seeds**

**Appendix $D_3$: *Xylopia aethiopica* powder**

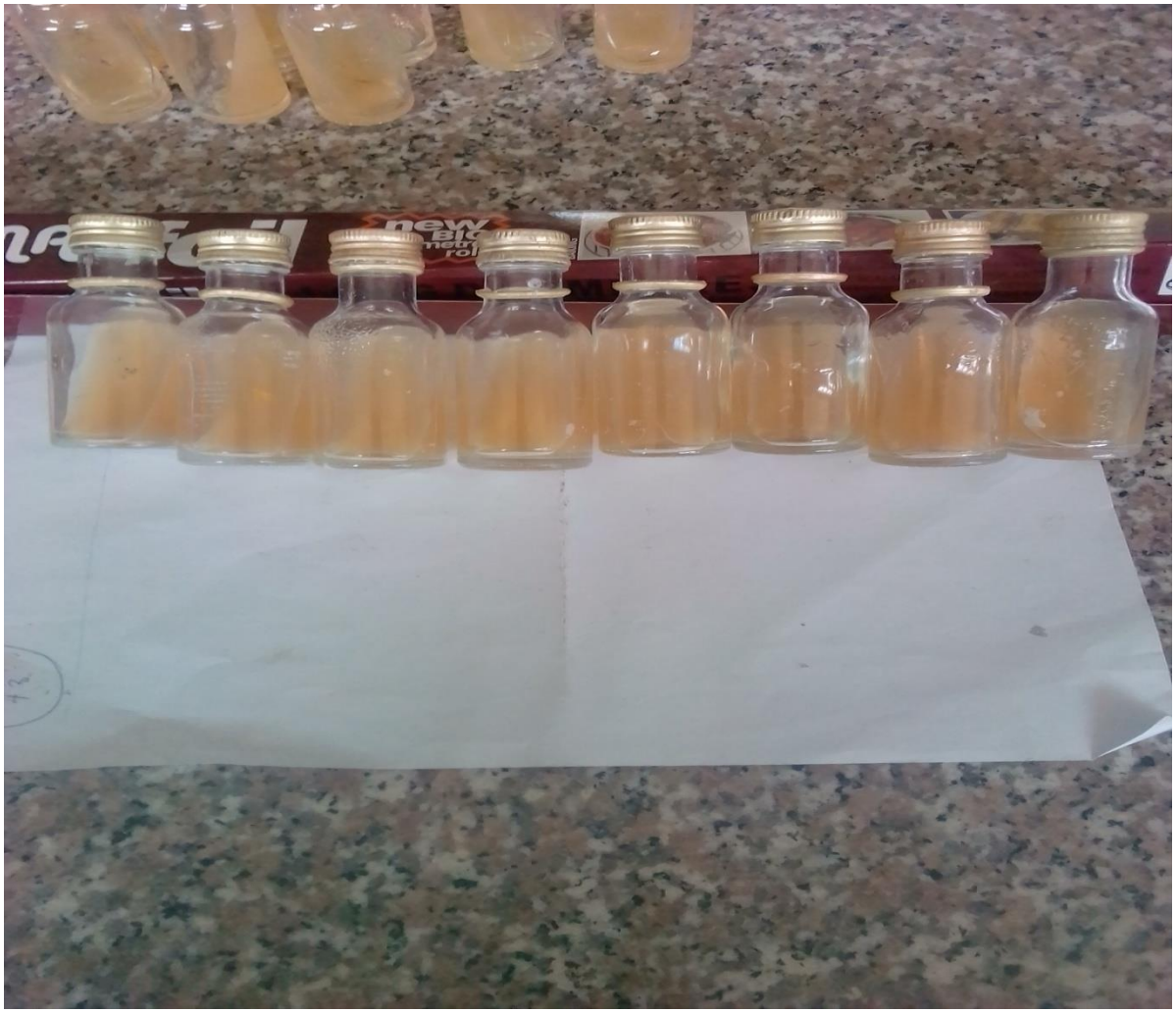
**Appendix $E_1$: Dried pods of *Piper guineense* spice**

**Appendix $E_2$: *Piper guineense* powder**

**Appendix F: Plates showing fungi growth cultured from tea samples**




**Appendix G: Slants used to preserve the fungi pure culture**



**Appendix H General nucleotide sequence for isolated fungi in tea and coffee**

1.

ATACTCTTGTACTTTACTTCCTNTTTTTACCAAAGAAAAAGATCCTGAGACCA
GCGTAATATTCCTGTCTAGCAAGCCAGACAGAAAATCACACACATTTTAGGTG
CTCACTGTAATAAAACAGCGATGCGACCCATTACCACATAAACAAATGTTATG
TGTGGGTTTGTGATGATACTGAAGCAGGCGTACTCTATAGAAAAACCATAGA
GTGCAAGCTGCGTTCAAAGACTCGATGAATCACTGAATATGCAATTCACACTA
GTTATCGCACTTTGCTACGTTCTTCATCGATGCGAGAACCAAGAGATCCATTG
TTAAAAGTTGTTTTTTATTAAACTTTATAATACTGAATTTCTAGGTTTATTATG
AAGGGTGCTCCTGAAACCAGGAGTGGCATCGATCAAACCCCAGATAGGTCTA
CCCATGACCAGTCTGAGTCTCTCAGCCAAATTTTCACAGTGTAGAAGCAATCA
CTTACCCCAGAGGAAAC

Rhizopus delemar (98.38%)- Rhizopus ajetea19

2.

TTGTACTTTACTTCCTNTTTTTACCAAAGAAAAAGATCCTGAGACCAGCGTAATATTCCTGTC

CAGAAAATCACACACATTTTAGGTGCTCACTGTAATAAAACAGCGATGCGACCCATTACCAC

TATGTGTGGGTTTGTGATGATACTGAAGCAGGCGTACTCTATAGAAAAACCATAGAGTGCAA

AGACTCGATGAATCACTGAATATGCAATTCACACTAGTTATCGCACTTTGCTACGTTCTTCAT

CAAGAGATCCATTGTTAAAAGTTGTTTTTTATTAAACTTTATAATACTGAATTTCTAGGTTTA

CCTGAAACCAGGAGTGGCATCGATCAA

Rhizopus delemar (98.00%) -Rizopus ajetea2019

3.

TTAGTTTNAAAGTTGCTGGATTATACTCTTGTACTTTACTTCCTNTTTTTACCA
AAGAAAAAGATCCTGAGACCAGCGTAATATTCCTGTCTAGCAAGCCAGACAG
AAAATCACACACATTTTAGGTGCTCACTGTAATAAAACAGCGATGCGACCCAT
TACCACATAAACAAATGTTATGTGTGGGTTTGTGATGATACTGAAGCAGGCGT
ACTCTATAGAAAAACCATAGAGTGCAAGCTGCGTTCAAAGACTCGATGAATC
ACTGAATATGCAATTCACACTAGTTATCGCACTTTGCTACGTTCTTCATCGATG

CGAGAACCAAGAGATCCATTGTTAAAAGTTGTTTTTTATTAAACTTTATAATA
CTGAATTTCTAGGTTTATTATGAAGGGTGCTCCTGAAACCAGGAGTGGCATCG
ATCAAACCCCAGATAGGTCTACCCATGACCAGTCTGAGTCTCTCAGCCAAATT
TTCACAGTGTAGAAGCAATCACTTACCCCAGAGGAAACCCTAAGAGGTAAGG
CGCTTTAACATAATTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTT
ACGACTTTTACTTCCAA

Rhizopus delemar UICC 524 LC514332.1
Rhizopus delemar UICC 121 (97.65%) – Rhizopus arrhizus strain ajetea3

4.
AACAGNGGGTTTCCTACCAAGATTTGAGGTCGAGCTTTTTGTTGTCTCGCAAC
ACTCGCTCTCGGCCGCCAAGCGTCCCTGAAAAAAGTCTAGTTCGCTCGGCCAG
CTTCGCTCCCTTTCAGGCGAGTCGCAGCTCCGACGCTCTTTACACGTCGTCCGC
TCCGCTCCCCCAACTCTGCGCACGCGCAAGATGGAAACGACGCTCAAACAGG
CATGCCCCCCGGAATGCCGAGGGGCGCAATGTGCGTTCAAGAACTCGATGAT
TCACGATGGCTGCAATTCACACTAGGTATCGCATTTCGCTGCGCTCTTCATCG
ATGCGAGAACCAAGAGATCCGTTGTTGAAAGTTTTGTTTGTTTTTCCGAAAAT
TTCCCTGGCCAACAATTGGCAAATTCCCCATTTTAAGGGGTGGTGGTTTCCTTC
CCCGCCCCCCCAGGGAATAATAAAACCCAAGAATGGACCCTCCCCCAGGTCC
CCCAACGAAACCCTGGTACCAACTTTAACTTCAAACTACCAAGATTTGAGGTC
GAGCTTTTTGTTGTCTCGCAACACTCGCTCTCGGCCGCCAAGCGTCCCTGAAA
AAAGTCTAGTTCGCTCGGCCAGCTTCGCTCCCTTTCAGGCGAGTCGCAGCTCC
GACGCTCTTTACACGTCGTCCGCTCCGCTCCCCCAACTCTGCGCACGCGCAAG
ATGGAAACGACGCTCAAACAGGCATGCCCCCCGGAATGCCGAGGGGCGCAAT
GTGCGTTCAAGAACTCGATGATTCACGATGGCTGCAATTCACACTAGGTATCG
CATTTCGCTGCGCTCTTCATCGATGCGAGAACCAAGAGATCCGTTGTTGAAAG
TTTTGTTTGTTTTTCCGAAAATTTCCCTGGCCAACAATTGGCAAATTCCCCATT
TTAAGGGGTGGTGGTTTCCTTCCCCGCCCCCCCAGGGAATAATAAAACCCAAG
AATGGACCCTCCCCCAGGTCCCCCAACGAAACCCTGGTACCAAC

Pichia sp. strain PM11 1 (89.25%) -Pichia kudriavzevii strain ajetea

5.
TTTGGTTGNTTCCTTAAAAGATCTGAGGTCAACCTGGNNAGAATGGTTGGAAA
ACGTCGGCAGGCGCCGGCCAATCCTACAGAGGATGTGACAAAGCCCCATACG

CTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGAG
AGGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGC
TCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGA
CTCGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCGT
TCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTG
CATTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTCTC
CGGCGGGCACGGGCCCGGGGGGCAGAGGCGCCCCCCCGGCGGCCGACAAGC
GGCGGGCCCGCCGAAGCAACAGGGTACAATAGACACGGATGGGAGGTTGGG
CCCAAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGAAAC
CTTGTTACGACTTTTACTTCCAA

Aspergillus niger clone bandF8 i EF175904.1

Aspergillus niger Aspergillus niger EF151435.1

(98.96%) - Aspergillus sp. isolate

8.
CCTGCCGTTAATTTCCTAACCATGATCCGAGGTCAACCTGGAAAGAATGGTTG
GAAAACGTCGGCANGCGCCGGCCAATCCTACAGAGCATGTGACAAAGCCCCA
TACGCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCC
GGAGAGGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATG
ACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCA
AAGACTCGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCT
GCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTG
ATTGCATTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGG
TCTCCGGCGGGCACGGGCCCGGGGGGCAGAGGCGCCCCCCCGGCGGCCGACA
AGCGGCGGGCCCGCCGAAGCAACAGGGTACAATAGACACGGATGGGAGGTT
GGGCCCAAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTACGG
AAACCTTGTTACGACTTTTTACTTCCAA

Penicillium georgiense isolate EV24

Aspergillus foetidus isolate BM13

(99.15%)- Aspergillus sp strain EF-1

r

9.
TTAATTTCCTAACCATGATCCGAGGTCAACCTGGAAAGAATGGTTGGAAAAC
GTCGGCANGCGCCGGCCAATCCTACAGAGCATGTGACAAAGCCCCATACGCT
CGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGAGA
GGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTC

GGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACT
CGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCGTTC
TTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCA
TTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTCTCCG
GCGGGCACGGGCCCGGGGGGCAGAGGCGCCCCCCCGGCGGCCGACAAGCGG
CGGGCCCGCCGAAGCAACAGGGTACAATAGACACGGATGGGAGGTTGGGCCC
AAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTAC.

Aspergillus niger isolate WIKO-NG MW680955.1

Aspergillus niger isolate WA-TKA MT628904.1

10.
AACCATGATCCGAGGTCAACCTGGAAAGAATGGTTGGAAAACGTCGGCANGC
GCCGGCCAATCCTACAGAGCATGTGACAAAGCCCCATACGCTCGAGGATCGG
ACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGAGAGGGGGACGGC
GACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTCGGACAGGCAT
GCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCA
CTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCGTTCTTCATCGATG
CCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCATTCAATCAAC
TCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTCTCCGGCGGGCACGG
GCCCGGGGGGCAGAGGCGCCCCCCCGGCGGCCGACAAGCGGCGGGCCCGCC
GAAGCAACAGGGTACAATAGACACGGATGGGAGGTTGGGCCCAAAGGACCC
GCACTCGGTAATGATCCTTCCGCAGGTTCACCTAC

Aspergillus niger isolate 8 MT588793.1

12.

ACAATGATTTCAGACAACGAGTTTGAAGATGGATCCTTTTTCCAATTGCTTTTTTTCTAATTGA

AATTTGATGCAAAGGAAAAAAAAAAACCTCAATTGATTTTTAATCCTATGGACTCATTTTCAG

AAGGTTTCCCTTTGACATCCCAATCCCAATGGACATTTAATAAAAAATGTCATTTGGGGGTTT

TGTACTGAAACAAGCATACCCCAAGGACAAGCCAAAGGGGGCAAGGGGCGTTCAAAAACTC

TTCGCAAAATGCAAATCGCACTAATTATCGCAATTTGCTACGCTCTTCATCGATGCGAGAGC

CCATTGCTTAAAGTTGTTTAAAAAAGTCCAAAGTCTCAATTGAAAGCCTTCTTTCTTTTTTTA

TCAATTAAAAATCAATTGGTATTAAAAAAAAATGTTTTTAATTGAAAATCCATTCTTTGTTAA

Syncephalastrum racemosum KP067274.1

(82.32% ) -Syncephalastrum monosporun strain NRRL

14.
NNTGCGANGGGTTCCTTACCATGAATCCGAGGTCAACCTGGGAAAGAATGGT
TGGAAAACGTCGGCAGGCGCCGGCCAATCCTACAGAGCATGTGACAAAGCCC
CATACGCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCC
CCGGAGAGGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAA
TGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTT
CAAAGACTCGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTC
GCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAA
CTGATTGCATTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTG
GGGTCTCCGGCGGGCACGGGCCCGGGGGGCAGAGGCGCCCCCCCGGCGGCCG
ACAAGCGGCGGGCCCGCCGAAGCAACAGGGTACAATAGACACGGATGGGAG
GTTGGGCCCAAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTAC
GGAAACCTTGTTACGACTTTTTACTTCCA

Aspergillus niger isolate WA-TKA
Aspergillus niger strain 7M1
(98.83%)-Penicillium georgiense isolate EV-24

15.
ACCGTTGGTTTCTACCAATGATTTGAGGTCGAGCTTTTTGTTGTCTCGCAACAC
TCGCTCTCGGCCGCCAAGCGTCCCTGAAAAAAAGTCTAGTTCGCTCGGCCAGC
TTCGCTCCCTTTCAGGCGAGTCGCAGCTCCGACGCTCTTTACACGTCGTCCGCT
CCGCTCCCCCAACTCTGCGCACGCGCAAGATGGAAACGACGCTCAAACAGGC
ATGCCCCCCGGAATGCCGAGGGGCGCAATGTGCGTTCAAGAACTCGATGATT
CACGATGGCTGCAATTCACACTAGGTATCGCATTTCGCTGCGCTCTTCATCGA
TGCGAGAACCAAGAGATCCGTTGTTGAAAGTTTTGTTTGTTTTTTCAAAAATTT
CCCTGGCCAACAATTTGCTATATCCCACTTTTTGGGGGTGGTTGTTTTCGTCCC
GCTCACGCAGGGAATAAATAAATCAAATAATGGATCCTTCCGCAGGTCCACC
TACGGAAACTTTGTACCAACTTTNCCATCAAA

Pichia kudriavzevii i
Pichia sp. strain PM11
(95.96%), (95.04%)

16.
TCCNTTGGTTCCTACCATGATCCGAGGTCAACCTGGGAAAAATGGTTGGAAAA
CGTCGGCAGGCGCCGGCCAATCCTACAGAGCATGTGACAAAGCCCCATACGC
TCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGAGA
GGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTC
GGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACT
CGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCGTTC
TTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCA
TTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTCTCCG
GCGGGCACGGGCCCGGGGGGCAAAGGCGCCCCCCCGGCGGCCGACAAGCGG
CGGGCCCGCCGAAGCAACAGGGTATAATAGACACGGATGGGAGGTTGGGCCC
AAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTT
GTTACGACTTTTTACTTCCAA

Gliocladium cibotii

Aspergillus tubingensis isolate HG6 i
(99.15%)- Aspergillus tubingensis strain 7-unknown
17.
TTCAANGGGTTCCTACCATGAATCCGAGGTCAACCTGGAAAGAATGGTTGGA
AAACGTCGGCAGGCGCCGGCCAATCCTACAGAGCATGTGACAAAGCCCCATA
CGCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGG
AGAGGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGAC
GCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAA
GACTCGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGC
GTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGAT
TGCATTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTC
TCCGGCGGGCACGGGCCCGGGGGGCAGAGGCGCCCCCCCGGCGGCCGACAA
GCGGCGGGCCCGCCGAAGCAACAGGGTACAATAGACACGGATGGGAGGTTG
GGCCCAAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGA
AACCTTGTTACGACTTTTACTTCCA

Aspergillus niger
(98.50%)-Aspergillus sp isolate UH 1552 020

18.
TCAGTTGGTTCCTTACCTGAATCCGAGGTCAACCTGGGAAAGAATGGTTGGAA
AACGTCGGCAGGCGCCGGCCAATCCTACAGAGCATGTGACAAAGCCCCATAC
GCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGA
GAGGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACG
CTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAG
ACTCGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCG
TTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATT
GCATTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTCT
CCGGCGGGCACGGGCCCGGGGGGCAGAGGCGCCCCCCCGGCGGCCGACAAG
CGGCGGGCCCGCCGAAGCAACAGGGTACAATAGACACGGATGGGAGGTTGG
GCCCAAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGAA
ACCTTGTTACGACTTTTACTTCCAA

Aspergillus niger clone bandF8 i EF175904.1
(99.33%)

20.
CCCNTTGTTTCCACCTTGACCTTCAGGATCATTAGATTTGAAAGTTGCTGGATT
ATACTCTTGTACTTTACTTCCTGGGCGAACCAAAGAAAAAGATCCTGAGACCA
GCGTAATATTCCTGCCTAGCAAGCCAGACAGAAAATCACACACATTTTAGGTG
CTCACTGTAATAAAACAGCGATGCGACCCATTACCACATAAACAAATGTTATG
TGTGGGTTTGTGATGATACTGAAGCAGGCGTACTCTATAGAAAAACCATAGA
GTGCAAGCTGCGTTCAAAGACTCGATGATTCACTGAATATGCAATTCACACTA
GTTATCGCACTTTGCTACGTTCTTCATCGATGCGAGAACCAAGAGATCCATTG
TTAAAAGTTGTTTTTTATTAAACTTTATAATACTGAATTTCTAGGTTTATTATG
AAGGGTGCTCCTGAAACCAGGAGTGGCATCGATCAAACCCCAGATAGGTCTA
CCCATGACCAGTCTGAGTCTCTCAGCCAAATTTTCACAGTGTAGAAGCAATCA
CTTACCCCAGAGGAAACCCTAAGAGGTAAGGCGCTTTAACATAATTAATGAT
CCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTACTTCCA

Rhizopus delemar UICC 524
Rhizopus delemar UICC 121

21.

TTAATTTCCTAACCATGATCCGAGGTCAACCTGGAAAGAATGGTTGGAAAAC
GTCGGCANGCGCCGGCCAATCCTACAGAGCATGTGACAAAGCCCCATACGCT
CGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGAGA
GGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTC
GGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACT
CGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCGTTC
TTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCA
TTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTCTCCG
GCGGGCACGGGCCCGGGGGGCAGAGGCGCCCCCCCGGCGGCCGACAAGCGG
CGGGCCCGCCGAAGCAACAGGGTACAATAGACACGGATGGGAGGTTGGGCCC
AAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTAC

Aspergillus niger isolate WIKO-NG MW680955.1

(99.64%), (99.46% - A.niger isolate WA- TKA), (A. foetidus isolate EL S6)
22.
CATGTGACAAAGCCCCATACGCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCC
GGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTCGGACAGGCA
TACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCAATTCACATT
CTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCATT
TGCACGCTTTCAGACAGTGTTCGTGTTGGGGTCTCCGGCGGGCACGGGCCCGGGGGGCAGAC
CGGCCGACAAGCGGCGGGCCCGCCGAAGCA

Aspergillus foetidus isolate BM13 MK91

23.
GAAAAATGGTTGGAAAACGTCGGCAGGCGCCGGCCAATCNNTTNAGCATGTGACAAAGCCC
GATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGAGAGGGGGACGGCGAC
CCGGGCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGC
AAGACTCGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCGTTCTTC
CCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCATTCAATCAACTCAGACTGCACGCTTT
TGTTGGGGTCTCCGGCGGGCACGGGCCCGGGGGGCAAAGGCGCCCCCCCGGCGGCCGACAA
CCGAAGCAACAGGGTATAATAGACAC
Fungal sp. isolate Aspergillus niger strain T-A7
(98.73%), (98.72% - A. sp strain 8 unknown)

24.
AAAANNGGTTGGAAAACGTCGGCAGGCGCCGGCCAATNNNTTNAGNATGTGACAAAGCCCC

AGGATCGNANNTNNTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGAGAGGGGGACGGCGA
CAAGCCGGGCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGG
CGTTCAAAGACTCGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCG
CCGGAACCAAGAGATCCATTGTTGAAAGTTTTAA

Aspergillus welwitschiae isolate 1017
(96.21%), (95.05% - A. sp strain 13 unknown)

25.
CCCNTTGTTTCTTCCTGCATCCGAGGTACAAGCCTGTGGGAAGAATGGTTGGA
AAATGTCGGCNNGCGCCGGCCAATCCTACAGAGCATGTGACAAAGCCCCATA
CGCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGG
AGAGGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGAC
GCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAA
GACTCGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGC
GTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGAT
TGCATTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTC
TCCGGCGGGCACGGGCCCGGGGGGCAGAGGCGCCCCCCCGGCGGCCGACAA
GCGGCGGGCCCGCCGAAGCAACAGGGTACAATAGACACGGATGGGAGGTTG
GGCCCAAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGA
AACCTTGTTACGACTTTTACTTCAA

Aspergillus niger isolate 8, MT588793.1

(98.46%), (98.12% - A. sp isolate UH 1552 020)

26.
TTAATTTCCTAACCATGATCCGAGGTCAACCTGGAAAGAATGGTTGGAAAAC
GTCGGCANGCGCCGGCCAATCCTACAGAGCATGTGACAAAGCCCCATACGCT
CGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGAGA
GGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTC
GGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACT
CGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCGTTC
TTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCA
TTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTCTCCG
GCGGGCACGGGCCCGGGGGGCAGAGGCGCCCCCCCGGCGGCCGACAAGCGG
CGGGCCCGCCGAAGCAACAGGGTACAATAGACACGGATGGGAGGTTGGGCCC
AAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTAC

Aspergillus niger isolate RMUAN75 MT550026.1

(99.46%), (99.29% - A. foetidus EL S6)

27.
CCCGTTGTTTCTANCAATGATCCGAGGTCAACCTGGAAAGAATGGTTGGAAA
ACGTCGGCANGCGCCGGCCAATCCTACAGAGCATGTGACAAAGCCCCATACG
CTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGAG
AGGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGC
TCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGA
CTCGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCGT
TCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTG
CATTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTCTC
CGGCGGGCACGGGCCCGGGGGGCAGAGGCGCCCCCCCGGCGGCCGACAAGC
GGCGGGCCCGCCGAAGCAACAGGGTACAATAGACACGGATGGGAGGTTGGG
CCCAAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGAAAC
CTTGTTACGACTTTTTACTTCCAA

Aspergillus niger isolate MZC-14 C0695
(99.66%), (99.48% A. sp strain EF-1)

28.
TCCTTGGTTCCTTCCATGAATCCGAGGTCAACCTGGGAAAAATGGTTGGAAAA
CGTCGGCAGGCGCCGGCCAATCCTACAGAGCATGTGACAAAGCCCCATACGC
TCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGAGA
GGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTC
GGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACT
CGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCGTTC
TTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCA
TTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTCTCCG
GCGGGCACGGGCCCGGGGGGCAAAGGCGCCCCCCCGGCGGCCGACAAGCGG
CGGGCCCGCCGAAGCAACAGGGTATAATAGACACGGATGGGAGGTTGGGCCC
AAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTT
GTTACGACTTTTTACTTCCN

Aspergillus sp. voucher jxbr-03
(99.83%), (99.48% A. sp isolate BSSF-3)

29.

CCTTCCATGAATCCGAGGTCAACCTGGGAAAAATGGTTGGAAAACGTCGGCAGGCGCCGGC
GAGCATGTGACAAAGCCCCATACGCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGC
CCGGAGAGGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTCGC
CCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGC
GTTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTT
TTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTCTCCGGCGGGCACGC
CAAAGGCGCCCCCCCGGCGGCCGACAAGCGGCGGGCCC

Aspergillus sp. isolate AUMS56
(A.sp isolate SO 4-7)

30.
ATGTGACAAAGCCCCATACGCTCGAGGATCGGACGCGGTGCCGCCGCTGCCT
TTCGGGCCCGTCCCCCCGGAGA
GGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTC
GGACAGGCATGCCCCCCGGAA
TACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCA
ATTCACATTAGTTATCGCATTTC
CTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAAC
TGATTGCATTCAATCAACTCAGAC
TGCACGCTTTCAGACAGTGTTCGTGTTGGGGTCTCCGGCGGGCACGGGCCCGG
GGGGCAGAGGCGCCCCCCCGG
CGGCCGACAAGCGGCGGGCCCGCCGAAG

Aspergillus foetidus isolate BM13 MK91

(99.75% - Aspergillus foetidus isolate TW 1.2)

31.
CCCGTTNTTTCTACAATGATTTCAGACAACGAGTTTGAAGATGGATCCTTTTTC
CAATTGCTTTTTTTCTAATTGAAAAAAATTTGATGCAAAGGAAAAAAAAAACC
TCAATTGATTTTTAATCCTATGGACTCATTTTCAGGATGAAAGGTTTCCCTTTG
ACATCCCAATCCCAATGGACATTTAATAAAAAATGTCATTTGGGGGTTTATAG
TTGTACTGAAACAAGCATACCCCAAGGACAAGCCAAAGGGGGCAAGGGGCGT
TCAAAAACTCGATGATTCGCAAAATGCAAATCGCACTAATTATCGCAATTTGC
TACGCTCTTCATCGATGCGAGAGCCAAGAGATCCATTGCTTAAAGTTGTTTAA
AAAAGTCCAAAGTCTCAATTGAAAGCCTTCTTTCTTTTTTTTATACTTCAATTC

AATTAAAAATCAATTGGTATTAAAAAAAAATGTTTTTAATTGAAAATCCATTC
TTTGTTAACCCAGGAATCGTTGCAATAAACGGGGGGAATACCAAATTCTTTCC
TCTCAATGAAGAGAGAAAAAAAAAATTGGAAAGGATCCTTCCGCAGGTTCCC
CTACGGAAACCTTGTTACAATTTTTACTTCAAA

Syncephalastrum racemosum LC097194.1

32.
GAGGTCACCTGGAAAAATGGTTGGAAAACGTCGGCAGGCGCCGGCCAATCNN
TTNAGCATGTGACAAAGCCCCATACGCTCGAGGATCGGACGCGGTGCCGCCG
CTGCCTTTCGGGCCCGTCCCCCCGGAGAGGGGGACGGCGACCCAACACACAA
GCCGGGCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAATAC
CAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCAATT
CACATTAGTTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGA
TCCATTGTTGAAAGTTTTAACTGATTGCATTCAATCAACTCAGACTGCACGCTT
TCAGACAGTGTTCGTGTTGGGGTCTCCGGCGGGCACGGGCCCGGGGGGCAAA
GGCGCCCCCCCGGCGGCCGACAAGCGGCGGGCCCGCCGAAGCAACAGGGTAT
AATAGACACGGATGGGAGGTTGGGCCCAAAGGACCCGCACTCGGTAATGATC
CTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTACTTCCA

Aspergillus tubingensis strain HM64
(98.78%)

33.
TACGCTCGAGGACGCTGCCTTTCGGGCCCGTCCCCCCGGAGAGGGGGACGGC
GACCCAACACACAAGCCGGGCTNATGANGCTCGGACAGGCATGCCCCCCGGA
ATACCNGGGGGNGCAATGNGCGTTCAAAGACTCGATGATTCANTGAATTCTG
NAANNNNNATTAGTTATCNCATTTCNCTGCGTTCTTCATCNATGCCGGANCCN
AGAGATCCNTNGNTGAANGNTNTANCTGATTGNNTTCAATCNACTCANACTG
NNNNCTTTCAGACAGTGTTCNNGTTGGGGNCTNCGGGGGGCAANNGCGCCCC
CNNNGNNGCCGACAAGCGGNGGGCCCGCCGANGGNATAATAGACACGGATG
GGANGNNGGGNCNNAAGGACCCGCACTCNGNNATGATCCNTCGNTCANCNN
NNNAAANNTNNNTNNNANTTTTNNNTCNNNN

Aspergillus sp. NCIM 947 KR261381.1

(85.19%),

34.

AGGACGCTGCCTTTCGGGCCCGTCCCCCCGGAGAGGGGGACGGCGACCCAACACACAAGCC

ANGCTCGGACAGGCATGCCCCCCGGAATACCNGGGGGNGCAATGNGCGTTCAAAGACTCGA

GAATTCTGNAANNNNNATTAGTTATCNCATTTCNCTGCGTTCTTCATCNATGCCGGANCCNA

GNTGAANGNTNTANCTGATTGNNTTCAATCNACTCANACTGNNNNCTTTCAGACAGTGTTCI

NCTNCGGGGGGCAANNGCGCCCCCNNNGN

Aspergillus sp. isolate HHELF3 MK862455.1

(84.72% A. sp isolate HHELF 3)

35.

NAGGTCACCTGGAAAANNGGTTGGAAAACGTCGGCAGGCGCCGGCCAATNN
NTTNAGNATGTGACAAAGCCCCATACGCTCGAGGATCGNANNTNNTGCCGCC
GCTGCCTTTCGGGCCCGTCCCCCCGGAGAGGGGGACGGCGACCCAACACACA
AGCCGGGCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAATA
CCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCAAT
TCACATTAGTTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAG
ATCCATTGTTGAAAGTTTTAACTGATTGCATTCAATCAACTCAGACTGCACGC
TTTCAGACAGTGTTCGTGTTGGGGTCTCCGGCGGGCACGGGCCCGGGGGGCA
GAGGCGCCCCCCCGGCGGCCGACAAGCGGCGGGCCCGCCGAAGCAACAGGG
TACAATAGACACGGATGGGAGGTTGGGCCCAAAGGACCCGCACTCGGTAATG
ATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTACTTCCAA

Aspergillus welwitschiae isolate 1046 1

(97.37%)

36.

CCCGTTATTTCCTTCCATGCATCCGACGTGTCAAGCTGTGGAAACGATTGATTT
GCGTTCGGCAAGCGCCGGCCGGGCCTACAGAGCGGGTGACAAAGCCCCATAC
GCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTGGGGCCCGTCCCCCCCGG
AGAGGGGACGACGACCCAACACACAAGCCGTGCTTGATGGGCAGCAATGACG

CTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAG
ACTCGATGATTCACGGAATTCTGCAATTCACACTAGTTATCGCATTTCGCTGC
GTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGAT
TGCGATACAATCAACTCAGACTTCACTAGATCAGACAGAGTTCGTGGTGTCTC
CGGCGGGCGCGGGCCCGGGGCTGAGAGCCCCCGGCGGCCATGAATGGCGGGC
CCGCCGAAGCAACTAAGGTACAGTAAACACGGGTGGGAGGTTGGGCTCGCTA
GGAACCCTACACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTG
TTACGACTTTTTACTTCCA

Aspergillus flavus strain RCBBR_AEANW2

(98.65%), (99.82% - A. sp strain ZMGL 1), (98.14% - A. flavus strain 176 1 B2)

37.
CGGGTGACAAAGCCCCATACGCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTGGGGCCC
GGAGAGGGGACGACGACCCAACACACAAGCCGTGCTTGATGGGCAGCAATGACGCTCGGAC
CCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACGGAATTCTGCAA
TTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTT
TACAATCAACTCAGACTTCACTAGATCAGACAGAGTTCGTGGTGTCTCCGGCGGGCGCGGGC
GAGCCCCCGGCGGCCATGAATGGCGGGCCCGCCGAAGCAACTAAG

Aspergillus flavus strain ND28
(100%), (99.75% - A. flavus strain 1A)

39.
TGGAAAAAATTTGNAAAANNTCNNTANGCGCCGGNCANATGTGACAAATCCC
CATACGCTCGAGGATNNNTTTTTNTGCCGCCNCTGCCTTTCGGGCCCGTCCCC
CCGGANAGGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAA
TGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTT
CAAAGACTCGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTC
GCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAA
CTGATTGCATTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTG
GGGTCTCCGGCGGGCACGGGCCCGGGGGGCAAAGGCGCCCCCCCGGCGGCCG
ACAAGCGGCGGGCCCGCCGAAGCAACAGGGTATAATAGACACGGATGGGAG
GTTGGGCCCAAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTAC
GGAAACCTTGTTACGACTTTTACTTCCA

Aspergillus niger strain HM81 s MT609916.1
(97.66%), (97.66% A.turbigensis strain G36)

40.
NCAAGGGNTTGGTTTCCTACATGAATTTGAGGTCGAGCTTTTTGTTGTCTCGC
AACACTCGCTCTCGGCCGCCAAGCGTCCCTGAAAAAAAGTCTAGTTCGCTCGG
CCAGCTTCGCTCCCTTTCAGGCGAGTCGCAGCTCCGACGCTCTTTACACGTCG
TCCGCTCCGCTCCCCCAACTCTGCGCACGCGCAAGATGGAAACGACGCTCAA
ACAGGCATGCCCCCCGGAATGCCGAGGGGCGCAATGTGCGTTCAAGAACTCG
ATGATTCACGATGGCTGCAATTCACACTAGGTATCGCATTTCGCTGCGCTCTT
CATCGATGCGAGAACCAAGAGATCCGTTGTTGAAAGTTTTGTTTGTTTTTTCG
AAAATTTCTCTGGCCAACAATATGCTATATTCCACATTTTAGGGGTTGTTGTTT
TCGTTCCGCTCACGCAGGGAAGAATAAAATCACAGTAATGATCCTTCCGCAG
GTTCACCTACGGAAACCTTGTTACAACTTTTACTTCAAA

Pichia kudriavzevii isolate E20663_ MK267579.1

(96.81%)

41.
TTTCCTACATGAATTTGAGGTCGAGCTTTTTGTTGTCTCGCAACACTCGCTCTCGGCCGCCAA
CGCGCAAGATGGAAACGACGCTCAAACAGGCATGCCCCCCGGAATGCCGAGGGGCGCAATG

Pichia kudriavzevii IFM 53500 LC389027.1

Pichia kudriavzevii IFM 53077_ LC389023.1

(99.37%)

TTAATTTCCTAACCATGATCCGAGGTCAACCTGGAAAGAATGGTTGGAAAAC
GTCGGCANGCGCCGGCCAATCCTACAGAGCATGTGACAAAGCCCCATACGCT
CGAGGATCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGAGA
GGGGGACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTC
GGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACT
CGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCGTTC
TTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCA
TTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTCTCCG
GCGGGCACGGGCCCGGGGGGCAGAGGCGCCCCCCCGGCGGCCGACAAGCGG
CGGGCCCGCCGAAGCAACAGGGTACAATAGACACGGATGGGAGGTTGGGCCC
AAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTAC

Aspergillus niger isolate WIKO-NG MW680955.1

Aspergillus niger isolate WA-TKA MT628904.1

(99.64%), (99.46%), (99.29% - A. foetidus isolate ELS6)

42.

GCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCATTCAATCAACTCA

ACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTCTCCGGCGGGCACGGGCCCGGGGGGCAGAGGCGCCCCCC

CGGCGGCCGACAAGCGGCGGGCCCGCCGAAGCAACAGGGTACAATAGACACGGATGGGAGGTTGGGCCCAAA

GGACCCGCACTCGGTAATGATCCTTCCGCA

Aspergillus niger isolate 8 MT588793.1

Aspergillus niger isolate RMUAN75 MT550026.1

(100% A.niger isolate F4), (100% A. niger isolate BSH-5), (99.60% - A. niger strain SPJ 22)

43.

AAGGGNTTGGTTTCCTACATGAATTTGAGGTCGAGCTTTTTGTTGTCTCGCAA
CACTCGCTCTCGGCCGCCAAGCGTCCCTGAAAAAAAGTCTAGTTCGCTCGGCC
AGCTTCGCTCCCTTTCAGGCGAGTCGCAGCTCCGACGCTCTTTACACGTCGTC
CGCTCCGCTCCCCCAACTCTGCGCACGCGCAAGATGGAAACGACGCTCAAAC
AGGCATGCCCCCCGGAATGCCGAGGGGCGCAATGTGCGTTCAAGAACTCGAT
GATTCACGATGGCTGCAATTCACACTAGGTATCGCATTTCGCTGCGCTCTTCA
TCGATGCGAGAACCAAGAGATCCGTTGTTGAAAGTTTTGTTTGTTTTTTCGAA
AATTTCTCTGGCCAACAATATGCTATATTCCACATTTTAGGGGTTGTTGTTTTC
GTTCCG

44.

AAGGGNTTGGTTTCCTACATGAATTTGAGGTCGAGCTTTTTGTTGTCTCGCAACACTCGCT

CTCGGCCGCCAAGCGTCCCTGAAAAAAAGTCTAGTTCGCTCGGCCAGCTTCGCTCCCTTT

CAGGCGAGTCGCAGCTCCGACGCTCTTTACACGTCGTCCGCTCCGCTCCCCCAACTCTGC

GCACGCGCAAGATGGAAACGACGCTCAAACAGGCATGCCCCCCGGAATGCCGAGGGGC

GCAATGTGCGTTCAAGAACTCGATGATTCACGATGGCTGCAATTCACACTAGGTATCGC

ATTTCGCTGCGCTCTTCATCGATGCGAGAACCAAGAGATCCGTTGTTGAAAGTTTTGTTTG

TTTTTTCGAAAATTTCTCTGGCCAACAATATGCTATATTCCACATTTTAGGGGTTGTTGTTT

TCGTTCCG

Pichia kudriavzevii MK298061.1

45.

AAAATTTGNAAAANNTCNNTANGCGCCGGNCANATGTGACAAATCCCCATACGCTCGA
GGATNNNTTTTTNTGCCGCCNCTGCCTTTCGGGCCCGTCCCCCCGGANAGGGGGACGGC
GACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCC
CGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCAA
TTCACATTAGTTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCAT
TGTTGAAAGTTTTAACTGATTGCATTCAATCAACTCAGACTGCACGCTTTCAGACAGTGT
TCGTGTTGGGGTCTCCGGCGGGCACGGGCCCGGGGGGCAAAGG

Aspergillus tubingensis strain HM64 MT609909.1 (96.70% A. tubigensis strain C84N)
Aspergillus niger isolate MEBP0052 MT597437.1 (96.70% A. niger isolate GXIMD01012)

(96.15% A. niger isolate FC)

**Appendix J: Ethical approval for sensory evaluation studies**

**INSTITUTE FOR ADVANCED MEDICAL RESEARCH AND TRAINING (IAMRAT)**
**College of Medicine, University of Ibadan**
**Director: Prof. IkeOluwapo O. Ajayi,**
MBBS (Ib), M. Cl.Sc., Ph.D, MD, FMCGP, FWACP
**Tel:** 08023268431
**E-mail:** ikeajayi2003@yahoo.com

UI/UCH EC Registration Number: **NHREC/05/01/2008a**

**NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW**

**Re: Mitigation of trichothecene contamination in tea (*camellia spp*) and coffee (*robusta spp*) using powder extract of selected tropical spices and its sensory attributes**

UI/UCH Ethics Committee assigned number: UI/EC/22/0087
Name of Principal Investigator: **Abiodun O. Ajewole**
Address of Principal Investigator: Department of Food Technology
University of Ibadan, Ibadan

Date of receipt of valid application: 18/03/2022

Date of meeting when final determination on ethical approval was made: **N/A**

This is to inform you that the research described in the submitted protocol, the consent forms, and other participant information materials have been reviewed and *given full approval by the UI/UCH Ethics Committee.*

This approval dates from **05/07/2022 to 06/07/2023.** If there is delay in starting the research, please inform the UI/UCH Ethics Committee so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the* UI/UCH EC *assigned number and duration of* UI/UCH EC *approval of the study.* It is expected that you submit your annual report as well as an annual request for the project renewal to the UI/UCH EC at least four weeks before the expiration of this approval in order to avoid disruption of your research.

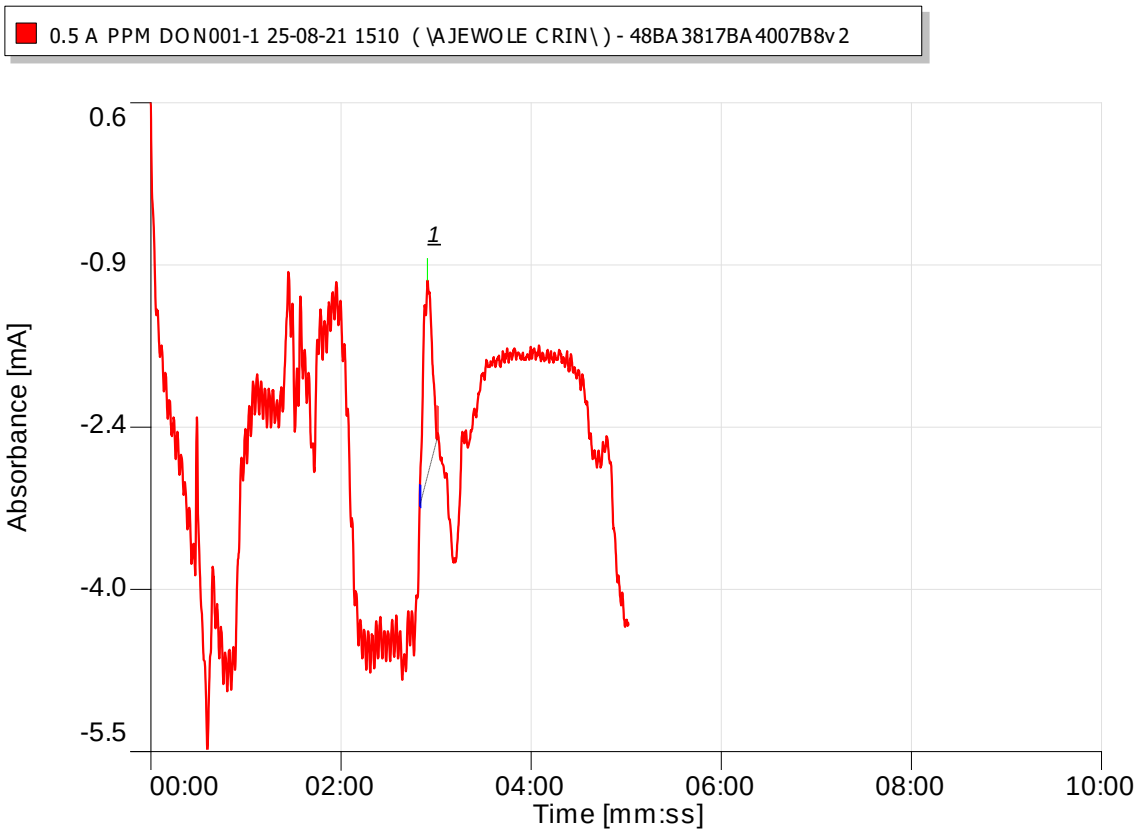
*The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the* UI/UCH EC. *No changes are permitted in the research without prior approval by the* UI/UCH EC *except in circumstances outlined in the Code. The* UI/UCH EC *reserves the right to conduct compliance visit to your research site without previous notification.*

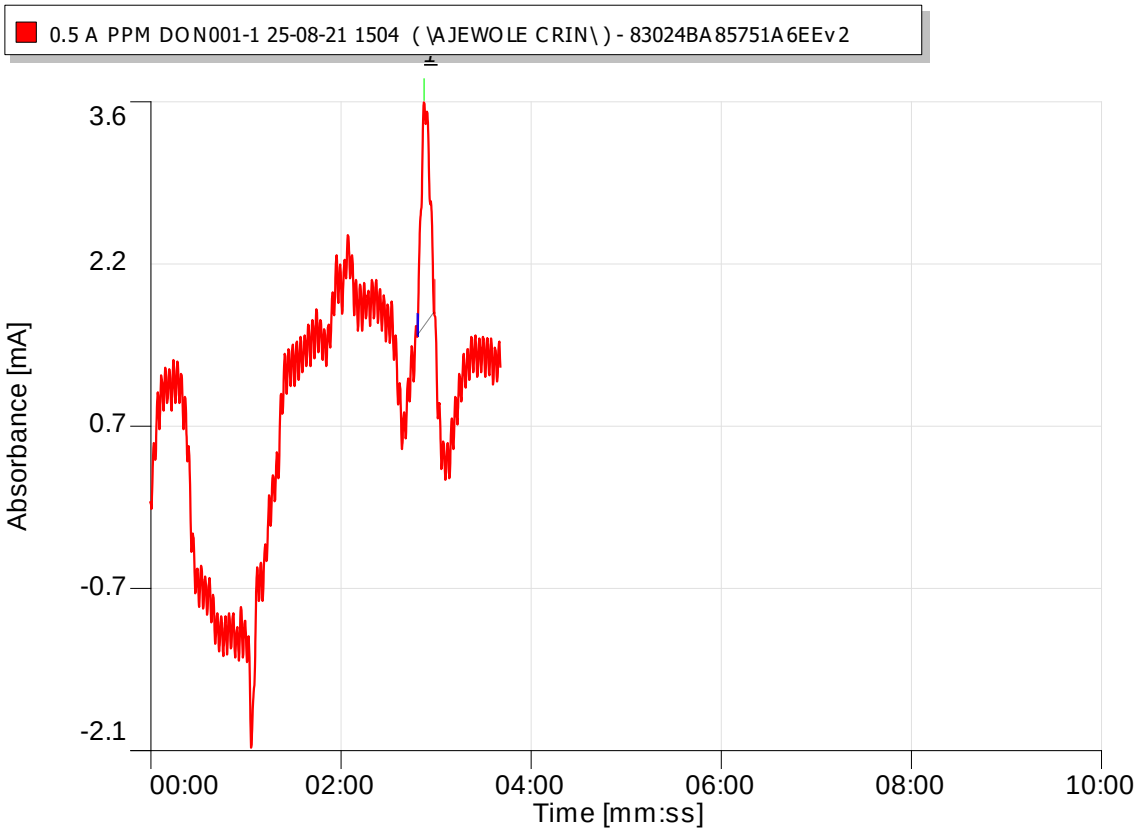
UI/UCH EC
05 JUL 2022

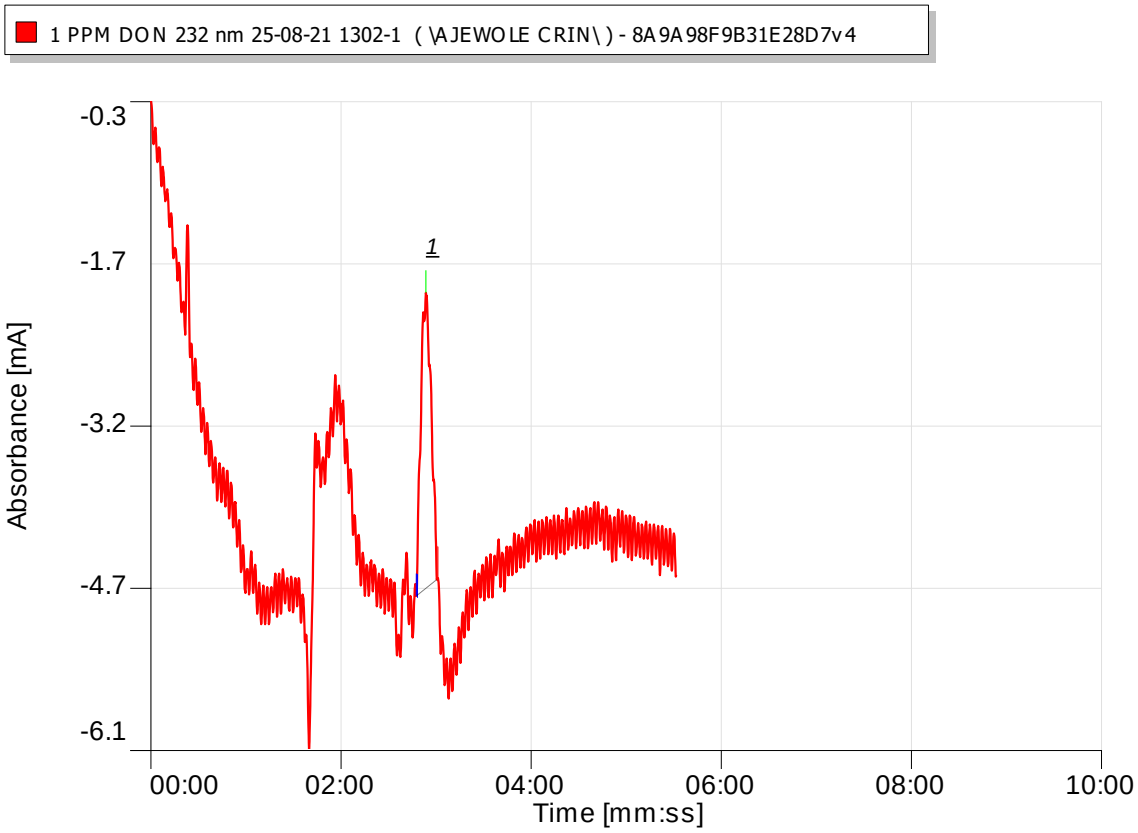
**Professor IkeOluwapo O. Ajayi**
Director, IAMRAT
Chairperson, UI/UCH Research Ethics Committee
E-mail: uiuchec@gmail.com

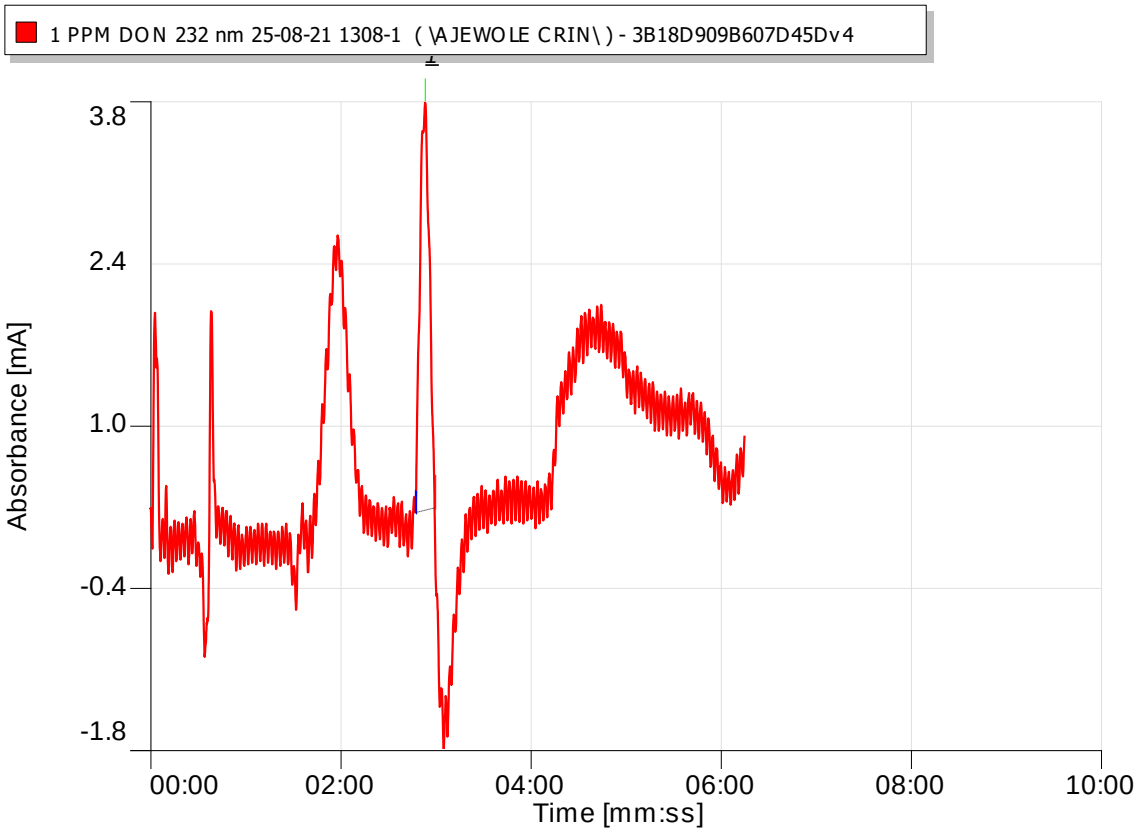
**Appendix I : HPLC used for fractional quantitation of mycotoxins in CRIN Laboratory**

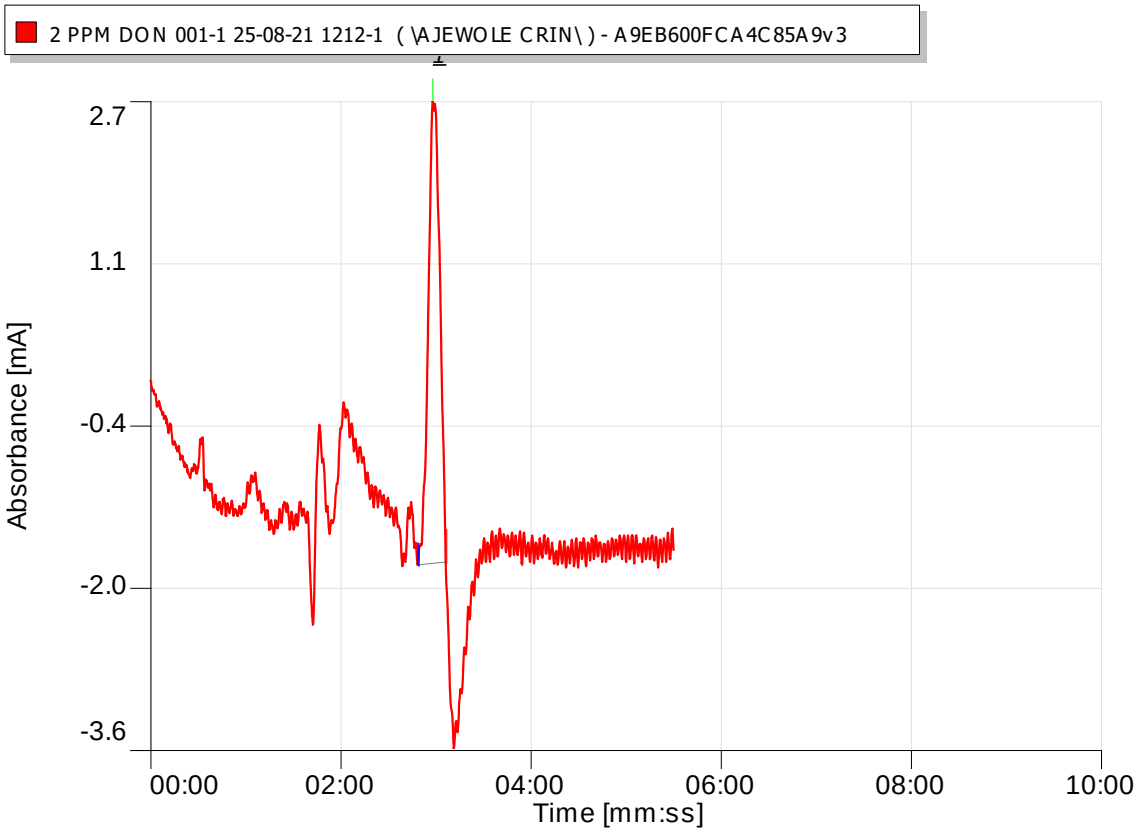
## Appendix J: HPLC chromatogram

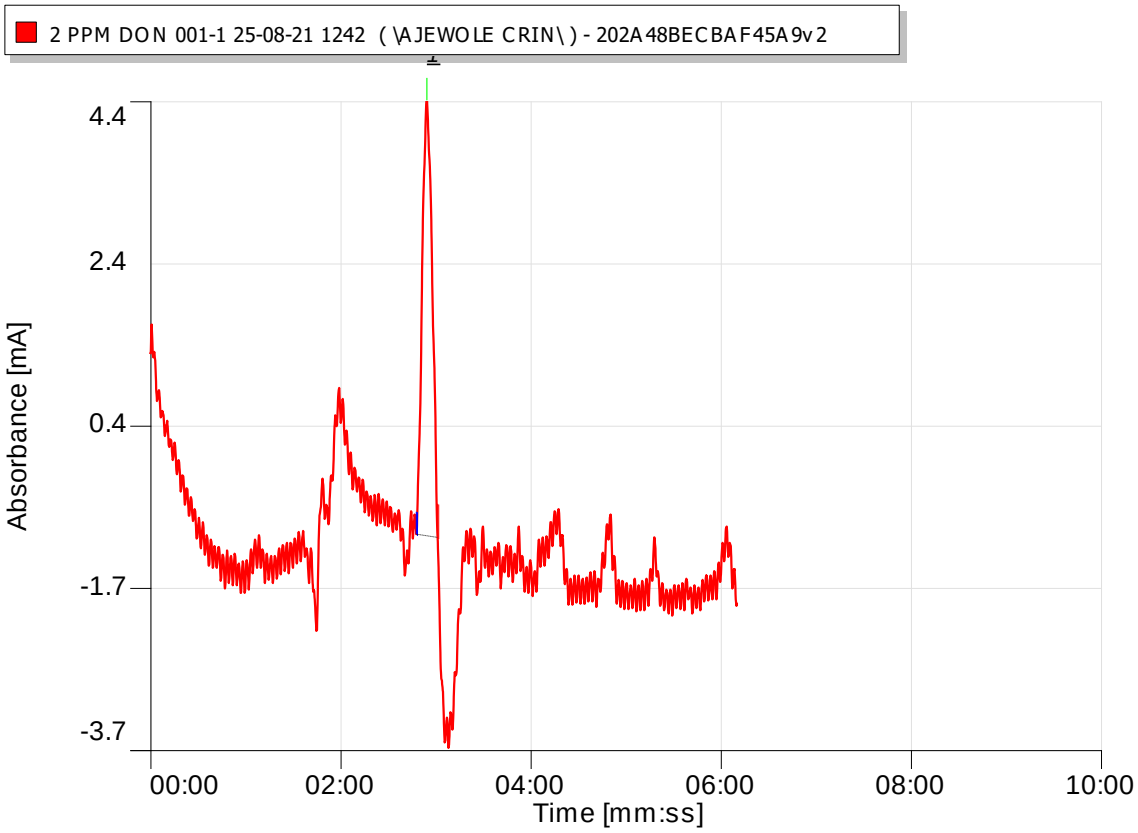


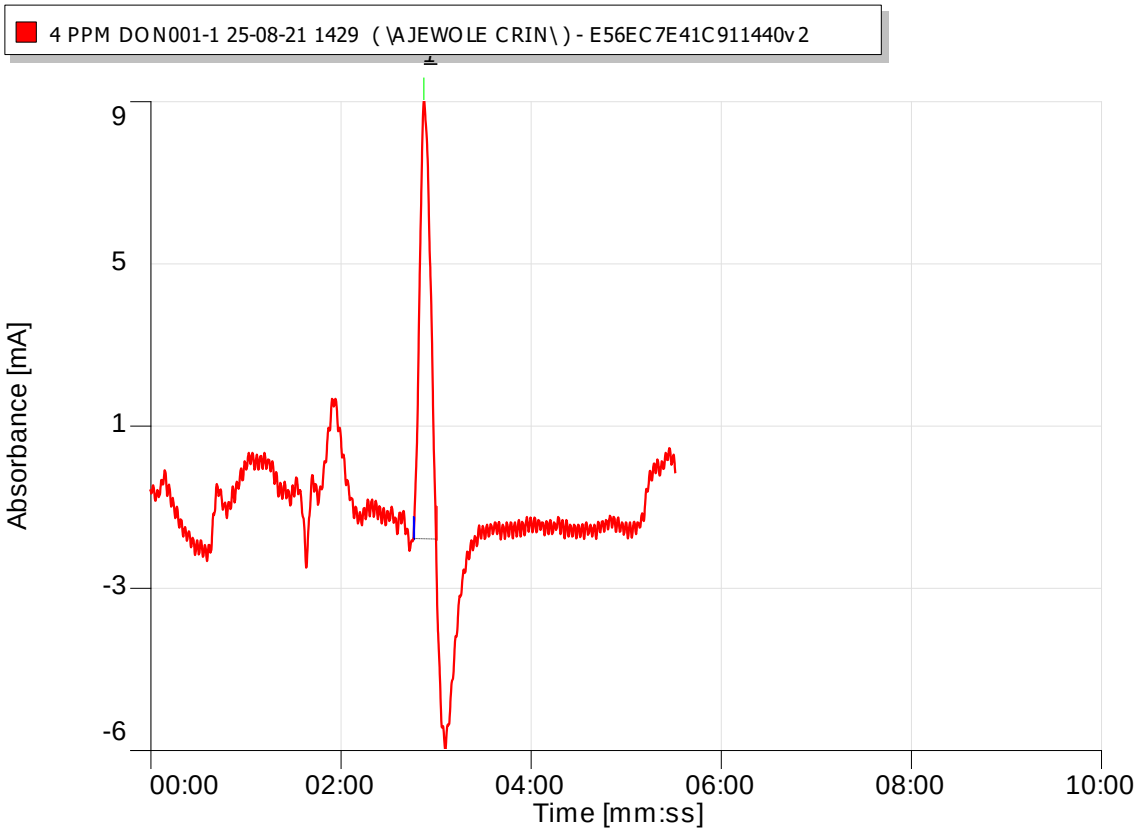

0.5 A PPM DON001-1 25-08-21 1504 ( \AJEWOLE CRIN\ ) - 83024BA85751A6EEv2
1
3.6
2.2
0.7
-0.7
-2.1
Absorbance [mA]
00:00
02:00
04:00
06:00
08:00
10:00
Time [mm:ss]

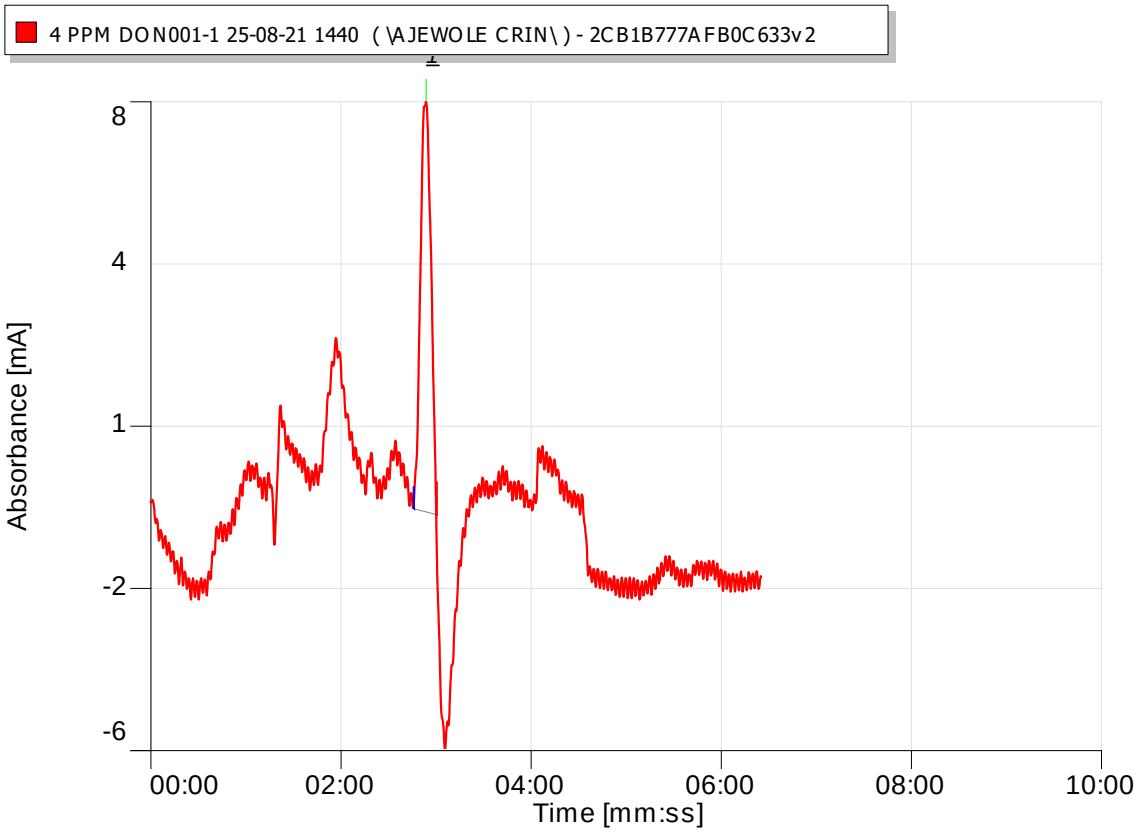

1 PPM DON 232 nm 25-08-21 1302-1 ( \AJEWOLE CRIN\ ) - 8A9A98F9B31E28D7v4
-0.3
-1.7
-3.2
-4.7
-6.1
1
Absorbance [mA]
00:00
02:00
04:00
06:00
08:00
10:00
Time [mm:ss]


1 PPM DON 232 nm 25-08-21 1308-1 ( \AJEWOLE CRIN\ ) - 3B18D909B607D45Dv4
3.8
2.4
1.0
-0.4
-1.8
Absorbance [mA]
00:00
02:00
04:00
06:00
08:00
10:00
Time [mm:ss]


2 PPM DON 001-1 25-08-21 1212-1 ( \AJEWOLE CRIN\ ) - A9EB600FCA4C85A9v3
2.7
1.1
-0.4
-2.0
-3.6
Absorbance [mA]
00:00
02:00
04:00
06:00
08:00
10:00
Time [mm:ss]


2 PPM DON 001-1 25-08-21 1242 ( \AJEWOLE CRIN\ ) - 202A48BECBAF45A9v2
4.4
2.4
0.4
-1.7
-3.7
Absorbance [mA]
00:00
02:00
04:00
06:00
08:00
10:00
Time [mm:ss]


4 PPM DON001-1 25-08-21 1429 ( \AJEWOLE CRIN\ ) - E56EC7E41C911440v2
9
5
1
-3
-6
Absorbance [mA]
00:00
02:00
04:00
06:00
08:00
10:00
Time [mm:ss]


4 PPM DON001-1 25-08-21 1440 (\AJEWOLE CRIN\) - 2CB1B777AFB0C633v2
Absorbance [mA]
8
4
1
-2
-6
00:00
02:00
04:00
06:00
08:00
10:00
Time [mm:ss]