Original Article *Journal of Sustainability and Environmental Management (JOSEM), Vol. 1, No. 4 (2022), 371 – 375*
p-ISSN 2822-1729, e-ISSN 2822-1737 Available online at https://www.nepjol.info/index.php/josem

Determination of Aflatoxin Levels and Prevalence of Fungal Flora of Cwande Condiments Sold in Zuru Local Government Area, Kebbi State, Nigeria

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Abstract: Aflatoxins are group of secondary metabolites produced by certain mold species which are dangerous to humans and animals. Cwande is a local condiment that is used to add flavor to the food, it get infected with fungi and aflatoxins as a result of improper processing and storage procedures. This study aimed to determine the aflatoxin levels in Cwande condiments sold in Zuru Local Government Area, Kebbi State, Nigeria, as well as the prevalence of fungal flora. Twenty (20) dried processed samples from four different collection points in Zuru central market were chosen at random and placed in brand-new polythene bags. Fungi were isolated on Potato Dextrose Agar by Standard Dilution Plate method. Aflatoxin was determined using the ELISA method, which is enzyme-linked immunosorbent assay. Five fungal species were isolated and identified as *Aspergillus flavus, A. niger, A. fumigatus, Rhizopus stolonifer* and *Fusarium Oxysporum*. Fungal species were present in varying degrees, from 9.09% to 39.39%. Aflatoxins varied from 2.539 to 2.546 in all samples. These results led to the discovery that the commercially available Cwande in the Zuru central market was tainted with various fungal species, including aflatoxigenic ones. All of the samples tested positive for aflatoxin according to the analysis, however none of them had levels that exceeded the 10g/kg maximum permissible limit for humans stipulated by the EU and NAFDAC. More research should be conducted in order to determine the nutritional and anti-nutritional components of the regional condiment (Cwande).

Keywords: *Aflatoxin, Cwande, Fungal flora, Nigeria, Zuru LGA*

Conflicts of interest: None
Supporting agencies: None

Received 05.07.2022; Revised 06.09.2022; Accepted 14.09.2022

Cite This Article: Ahmad, A., Keta, J.N. & Singh, D. (2022). Determination of Aflatoxin Levels and Prevalence of Fungal Flora of Cwande Condiments Sold in Zuru Local Government Area, Kebbi State, Nigeria. *Journal of Sustainability and Environmental Management*, 1(4), 371-375.

1. Introduction

Due to the formation of poisonous byproducts known as mycotoxins, mold growth reduces the quality of food and poses a risk to human health. Systemic mycosis, cutaneous mycosis, subcutaneous mycosis, and infections of the ears and eyes are among the illnesses caused by several mycotoxin-producing organisms such Aspergillus, Fusarium, and Penicillum sp. Aflatoxin has generated a great deal of concern among both consumers and farmers ever since it was found in several feedstuffs. Farmers face a major financial danger from this poison (Gardener et al., 2012).

Aspergillus continues to be the most common local condiment contaminant among the prominent molds that have been identified to do so, along with Penicillium, Rhizopus, Eurotium, Cladosporium, Trichoderma, Mucor, and Stachybotrys (Hashem and Alamri, 2010).

A series of mycotoxins known as "aflatoxins" are produced by various Aspergillus species, including *Aspergillus flavus* and *Aspergillus parasiticus* (Creppy, 2002). Even though the presence of Aspergillus mold does not always mean that there has been a contamination with aflatoxin, there is undoubtedly a higher risk (Robertson, 2005). Because they are highly toxic and are carcinogenic, teratogenic, hepatotoxic, and mutagenic, aflatoxins pose a serious threat to human health. Aflatoxin B1 (AFB1), the most common aflatoxin known to science, is still present in food (Lee et al., 2004).

AFB1 is also the most toxic metabolite, capable of causing genotoxicity in blood and reproductive cells, as

well as other toxic situations (Ezekiel et al., 2011). Additionally, they are thought to be the cause of human liver cancer, notably in a number of underdeveloped nations where several common foods contain high levels of aflatoxins (Oranusi et al., 2013). Epidemiological data gathered in East Africa, the Philippines, and Thailand revealed a link between exposure to aflatoxins and the likelihood of developing liver cancer. Additionally, aflatoxins have been noted as possible biological weapons for contaminating food and water (Smith, 2004). There have been reports of this toxin contaminating several condiments worldwide (Iqbal et al., 2011). Condiments are typically produced in countries with tropical climates that feature high temperatures, humidity and rainfall (Martins et al., 2001; Gumel, 2022).

Condiments are fragrant vegetable ingredients that are used in very little amounts to enhance or change the flavor of food (Bokhari, 2007). In Nigerian households, a variety of condiments are frequently used in the kitchen. Despite the fact that these condiments are consumed on a daily basis, very few reports of mycobiota and aflatoxin contamination have been made (Oranusi et al., 2013).

Due to their antioxidant qualities, condiments are widely used in Nigeria to flavor food and as medicines. Condiments, on the other hand, are exposed to fungal spores and eventual aflatoxin contamination as a result of improper handling techniques, such as drying condiments on bare ground. Additionally, condiments are typically handled in a way that is unhygienic, favoring the growth of mold and the formation of aflatoxin (Oranusi et al., 2013).

The Zuru people of Kebbi State, Nigeria, use the locally manufactured condiment (Cwande) as one of the most crucial soup ingredients. For many years, the locally made condiments has been cooked and consumed as a soup. Despite the fact that this condiment is crucial to the residents of the study area, little research has been done on Cwande. The isolation and identification of fungus linked to Cwande is one of the studies conducted in this area (Keta et al., 2019). Therefore, as fungi are known to create a poison known as mycotoxins, contamination of food and feed products like Cwande condiments by fungi can represent a major hazard to public health. Hence, this study aimed to determine the aflatoxin levels and prevalence of fungal flora of Cwande condiments sold in Zuru Local Government Area, Kebbi State, Nigeria.

2. Materials and methods

2.1. Sample collection

Twenty samples were randomly collected in new polythene bags from Zuru central market. The twenty samples were divided into four. Each condiment's five (5) replicates were mixed to prepare one composite sample, (Farid et al., 2013). This was done to prevent bias while collecting the samples because some were newly brought to the market while others had been in the market for a long time.

2.2. Isolation of fungal species

Potato Dextrose Agar (PDA) was prepared according to manufacturer's instructions and was autoclaved at 1210C pressure for 15 minutes to sterilize it. Using sterile forceps, four samples of each condiment were picked at random and placed on the Petridishes. The cultures were labeled, covered, and cultured for two to three days at room temperature. To produce pure cultures, mixed growth was subcultured into a freshly prepared medium (PDA) (Mukhtar et al., 2010). According to Lina (2013), the molds were recognized based on colonial look on culture plates.

2.3. Aflatoxin assay

Using a lab mill, individual Cwande samples were ground to a fine powder (Romer). To determine the amount of aflatoxin in the samples, an ELISA (AqraQuant Total Aflatoxin Assay 1/20) test kit was employed. This procedure was completed in three steps.

2.4. Sample extraction

The manufacturer's instructions for the Aqra Quant Total Aflatoxin Assay 1/20 test kit were followed while extracting the sample. Five (5g) of each grounded cwande sample were added to 25 mililiters of acetonitrile/water (84/16), and the solution was extracted by shaking for 30 minutes using an orbital shaker. The top layer of the extract was separated from the sample using a Whatman No. 1 filter paper, and the filtrate was then collected for cleanup.

2.5. Sample clean-up

MycoSep 226 aflazon was used to clean up the sample in accordance with the manufacturer's instructions in order to get rid of any contaminating elements like color and oil. Four milliliters of the extract were put into a glass tube, the top of which was then filled with a MycoSep column, which was pushed through until 0.5 milliliters of the purified extract were removed. The remaining 0.5 milliliters of the purified extract were then put into 2 milliliter vials and evaporated to dryness. With 0.5ml of 70/30 methanol/water, the residues in the vials were reconstituted and utilized for ELISA testing.

2.6. ELISA test

The AqraQuant Total Aflatoxin Assay 1/20 test kit handbook was followed in doing this. Each well with a green border was filled with 200 microliters of conjugate. Each standard and sample were each placed 100 microliters into the appropriate dilution, which had 200 liters of the conjugate already in it. After pipetting each well up and down three times to thoroughly mix the contents, 100 mL of each dilution well's contents were immediately placed into an associated antibody-coated microwell. After that, it was incubated for 15 minutes at room temperature. The water from the microwell strips

was dumped after each microwell had been washed by being filled with distilled water. The contents of the microwell strips were then discarded. There were a total of five washings done in this manner. After the sixth wash, microwell strips were tapped with absorbent paper towels to remove as much leftover water as feasible. Using a dry towel, the bottom of the microwells was dried. Each microwell received 100 microliter of the substrate, which was then added and left to develop for five minutes at room temperature. Each microwell strip received 100 microliters of stop solution, which caused the color to shift from blue to yellow. Using a microwell reader and a 450nm absorbance filter, the strips were read.

3. Results and discussion

3.1. Fungal contamination of the condiments

In this study, 33 isolates representing five species of 3 genera were isolated from Cwande samples collected. Aspergillus species were the most dominant fungi isolated. Among the species, it can be seen that *Aspergillus flavus* has the highest percentage 39.39% followed by *Aspergillus niger* with 24.24%, *Fusarium oxysporum* 15.15%, *Aspergillus fumigatus* 12.12% and *Rhizopus stolonifer* with 9.09% (Table 1).

3.2. Contamination of the condiments with aflatoxin

Aflatoxin was detected in all the Cwande samples, the concentrations of aflatoxin in the contaminated samples ranged from 2.539-2.546µg/kg (Table 2).

Table 1: The frequency of occurrence of isolated fungi from Cwande sample

Fungal isolates	Occurrence	Percentage (%)
Aspergillus flavus	13	39.39
Aspergillus niger	8	24.24
Rhizopus stolonifer	3	9.09
Fusarium oxysporum	5	15.15
Aspergillus fumigatus	4	12.12
Total	**33**	**100**

Table 2: Total Aflatoxin concentration from Cwande samples

Sample	Concentration	Fluorescence	Type of Aflatoxin
CW_1	2.542	Blue	Aflatoxin B
CW_2	2.546	Blue	Aflatoxin B
CW_3	2.539	Blue	Aflatoxin B

Key: CW = Cwande

4. Discussion

According to the study, the Cwande sample collected contained the fungi *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Aspergillus fumigatus*, and *Rhizopus stolonifer*. In contrast to the research by Keta et al. (2019), who isolated seven different fungal species, including *Graphium spp., Apophysomyces elegans, Cladophialophora bantiana, Pennicilium spp., Aspergillus flavus, Trichopyton spp*., and *Aspergillus niger*, this study revealed a decrease in the fungal load. The recent improvement in various techniques of processing, handling, and storage of the condiment may be the reason for the decrease in fungal load.

Aspergillus organisms were the easiest to trap because their spores are found in the air, Aspergillus had the highest frequency of occurrence in all the condiments

among the genera of fungi discovered in this investigation (Keta et al., 2019). The findings were essentially in agreement with those of several researchers studying various varieties of condiment mycobiota. For instance, Aspergillus was the most often isolated genus according to studies by Bokhari (2007) in Saudi Arabia and Sumanth et al. (2010) in India that identified fungal genera from tested condiments.

The findings also concur with those of Hashem and Alamri (2010), who found that A. flavus, A. parasiticus, and A. tamari were the three most common Aspergillus species previously recovered from a variety of condiments around the world, with A. flavus showing a higher frequency of occurrence. The lowest occurrence levels were seen in the other five fungal species, *A. fumigatus*, *Rhizopus stolonifer*, *Nigrospora sphaerica*, *Mucor hiemalis*, and *Rhizoctonia spp. A. fumigatus* was found in many condiments with a moderate to low frequency, according to multiple researches, including Abdulkadir et al. (2003). Both Farid et al. (2013) and Gnonlonfin et al. (2012) previously isolated the majority of these fungi from a variety of condiments.

All of the condiments in this investigation tested positive for total aflatoxin contamination, which ranged from 2.539 to 2.542 g/kg. However, no sample tested positive for aflatoxin contamination over the maximum allowable limit of 10 g/kg established by the EU and NAFDAC. This outcome is consistent with that reported by Haruna et al. (2016) in Katsina State, Nigeria, from several types of spices and condiments. Condiments may have become contaminated during processing, drying, storage, or even lengthy transportation from villages to cities. Additionally, it has a lot of nutrients that can help fungi flourish (Haruna et al., 2016).

5. Conclusion

The finding indicate that the Cwande that was commercially marketed in the Zuru central market was tainted with various fungal species, including aflatoxigenic ones. However, among the several isolated fungus, Aspergillus was the dominant genus. The samples contain aflatoxin at a low level that NAFDAC accepts, according to aflatoxin analysis.

Further research should be done to determine the nutritional and anti-nutritional content of the regional condiment (Cwande) due to the shortage of knowledge related to it (Cwande).

Acknowledgements

We acknowledge the National Agency for Food and Drug Administration and Control (NAFDAC), Kaduna, Dr. Almustapha Adamu Aliero, Head, Department of Microbiology, Kebbi State University of Science and Technology, Aliero, Dr. Ahmad Bagudo, Mal. Musa Isah Gumi, and Mal. Bashar Haruna Gulumbe for their technical assistance.

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