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Detection of Toxicogenic Molds in Some Legumes Sold in Local Markets of Ho, Ghana

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HIGHLIGHTS

- A total of 13 fungal species belonging to 7 genera were isolated from legume samples in Ghana.
- Toxicogenic fungal species belonged to the genera *Aspergillus*, *Fusarium*, and *Penicillium*.
- Low to moderate fungal counts of range 1.83 and 2.84 log CFU/g were recorded.
- Isolated fungi have probability to produce mycotoxins that cause adverse health effects in the consumers.

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Acronyms and abbreviations
CFU=Colony Forming Units
MC=Moisture Content

ABSTRACT

Background: Legumes are plants that contain edible seeds and belong to the family *Leguminosae* with varying nutritional benefits to humans and animals. This study aimed to detect and identify toxicogenic molds on some legumes purchased from two local markets in Ho Municipality, Ghana.

Methods: A total of 36 samples, including cowpea (n=9), soybean (n=9), brown bean (n=9), and Bambara bean (n=9) were randomly obtained from 2 local markets in the Volta region of Ghana. Culturing of the legume seeds were done on mycological media using serial dilution technique. Fungal species occurrence was also determined. Statistical Package for Social Sciences (SPSS) version 26 was used to analyze the data.

Results: Fungal counts on cowpea, soybean, brown beans, and Bambara beans ranged between 1.91 and 2.84 log Colony Forming Units (CFU)/g on both media. There were no statistically significant differences (p>0.05) in the samples from the different vendors. The Moisture Content (MC) ranged between 6.74 and 12.15%, pH ranged between 6.27±0.03-6.53±0.02. A total of 13 fungal species belonging to 7 genera were isolated on SDA and OGYEA media; *Aspergillus species* (*A. niger*, *A. terreus*, *A. flavus*, *A. fumigatus*, *A. ochraceus*, *A. parasiticus*), *Fusarium species (F. oxysporum)*, *Trichoderma harzianum*, *Rhizopus species (R. stolonifer)*, *Penicillium species (P. digitatum*, *P. verucosum)*, *Rhodotorula mucilaginosa*, and *Mucor racemosus* were recorded on the legumes.

Conclusion: The presence of some mycotoxigenic fungi in legumes examined in this study showed the potential health hazards in the local people of Ho, Ghana.

Introduction

Legumes are plants belonging to the *Leguminosae* family that contain seeds inside a capsule, also called *Fabaceae* (Kouris-Blazos and Belski, 2016; Staniak et al., 2014). Only a small portion of the 18,000 species that

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make up the huge family of climbers, herbs, shrubs, and trees known as *Leguminosae* are consumed as food by humans (Maphosa and Jideani, 2017). For subsistence farmers who cultivate legumes at the household level, they have shown to be a cheap supply of nutrition and a possible source of money. Legumes are an excellent source of important vitamins, minerals, unsaturated fats, and proteins for the human diet (Annor et al., 2014; Bouchenak and Lamri-Senhadji, 2013; Rebello et al., 2014). Peas, large beans, soybeans, green beans, and peanuts are common legumes that are known as grain legumes or food legumes used for human consumption. Recently, Mupunga et al. (2017) showed that groundnut (a legume) is used as a main ingredient in the Ready-to-Use Therapeutic Food (RUTF), an energy and protein-dense paste which meets the nutritional needs of the population at risk of malnutrition.

Legumes are grown all over tropical Africa as a cash crop mainly in Senegal, Gambia, Nigeria, Sudan, and Ghana (Senghor et al., 2020). In Ghana, they are grown in almost the 16 regions but predominate in the northern, upper East, upper West, Oti, and Volta regions where they are cultivated as cash crops (Oteng-Frimpong et al., 2015). It is estimated that the total production nationwide is about 193,200 metric tons from these regions of Ghana (Chakuri, 2018). In 2015, farmers in Ghana produced 417,000 metric tons from the pulse from 336,000 hectares of land (MoFA-SRID, 2016). Legumes are becoming more popular as people become more aware of their nutritional and health benefits (Maphosa and Jideani, 2017). In spite of these benefits that legumes provide, they are faced with so many challenges associated with their production, storage, and packaging in Ghana. A major problem of agricultural production is the loss of grains and seeds during and after harvest. Microorganisms, insects, and rodents contribute greatly to these postharvest losses. If cereals and legumes no treated properly during cultivation and storage, can expose humans to harmful naturally produced toxicants, such as microbial toxins, through their diet (Temba et al., 2017).

Globally, fungal contaminants are the main microorganisms responsible for food spoilage (Njobeh et al., 2009). Fungal contamination of food is recognized as a serious threat to food safety and security. Legumes are particularly susceptible to fungal contamination, therefore there is a need to assess, detect, and identify the fungal contamination. According to Embaby et al. (2013), during the stages of development, harvesting, and storage, numerous fungi attack legume seeds. Although more than 25 distinct fungus species have been found infesting stored grains and legumes (Duan et al., 2007), *Aspergillus*, *Penicillium*, and *Fusarium* species cause the majority of contamination and germ harm during storage. Food contamination by these fungal species and their subsequent mycotoxin production occur at different levels during the food production, processing, and storage of food (Embaby et al., 2013; Hanson et al., 2012). It should therefore be of major concern to reduce their existence in food by applying good agricultural practices. Also, fungi may reduce the consistency of cooking or baking and nutritional value, generate undesirable odors, and color, alter the appearance of stored food-grade grain, decrease germinability, and cause complete decay (Castillo et al., 2004).

So, this study sought to detect and identify molds on some legumes from two local markets in the Ho Municipality, Ghana.

Materials and methods

Study site

The Ho Municipality (Ghana) shares boundaries with Adaklu and Agotime-Ziope Districts to the South, Ho West District to the West, Hohoe Municipality to the North, and the Republic of Togo to the East. Two local markets in the Ho Municipality were selected based on the high patronage of legumes.

Sample collection

A total of 36 samples, including cowpea (n=9), soybean (n=9), brown bean (n=9), and Bambara bean (n=9) were randomly obtained from 2 local markets in the Volta region of Ghana in July-August 2020. Each sample type was collected from 3 points (vendors; Table 1). Approximately 100 g of each sample was collected and stored in sterile specimen containers (Nasco, USA) and transported in an ice chest freezer (Thermos 7,750, China) with cold packs at a temperature of 10 ^{o}C under aseptic conditions to the University of Health and Allied Sciences (UHAS) laboratory for microbiological analysis within 2 h of collection (Kortei et al., 2020).

Fungal analysis

Fungal analysis was carried out according to the procedure outlined by Kortei et al. (2018) and Odamtten et al. (2018). One g of each sample was transferred into 10 ml of sterile distilled water and soaked. All samples were weighed using an electronic balance (OHAUS®, Germany) with a readability of 0.01 g. One ml of each stock solution was serially diluted into 9 ml of sterile distilled water ten-fold up to 10^{-3}. One ml of each serial dilution was plated onto Sabouraud's Dextrose Agar (SDA) and Oxytetracycline Glucose Yeast Extract Agar (OGYEA) media plates prepared according to the manufacturer's instructions and incubated at 25 °C for 5-7 days.

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Enumeration was carried out by a colony counter. Fungal counts were recorded in standard form and later transformed into the logarithmic form. Colony Forming Units per gram (CFU/g) was calculated using the formula (Kortei et al., 2021).

CFU/g=(no. of colonies×reciprocal of the dilution factor)/volume of culture plate (1)

Percentage occurrence of fungal species was calculated using the formula:

$$\text{Percentage (\%) occurrence of fungal species} = \frac{\text{Number of fungal species}}{\text{Total number of fungi isolated}} \times 10 \quad (2)$$

A drop of Lactophenol Cotton Blue (LPCB) dye was placed on the slide; a sterile iron needle was used to transfer a tiny piece of a colony of LPCB on the slide. The colony was teased into very tiny pieces using an iron needle. The slide was covered with a coverslip with a magnification ×400 used. Identification of the fungi was done macroscopically (texture and color of the plate) and microscopically by observation of their cultural and morphological features (Table 2) under the microscope.

Molds and yeast that appeared were identified by their cultural and morphological characteristics using standard identification manuals (Samson et al., 2004; Samson and Van Reenen-Hoekstra, 1988) (Table 2).

Determination of Moisture Content (MC)

The MC of the legume samples used was determined. Five g of the crushed homogenate of the legume samples were weighed into petri dishes and dried overnight (16 h) in an oven at 105 °C. Cooling of petri dishes were done in a desiccator and the final weight recorded with an Accu Lab ALC-150.3, USA. The MC was determined using the following equation:

$$MC = \frac{W2 - W3}{W2 - W1} \times 100$$

Where; W1 is the weight of an empty petri dish

W2 is the weight of sample+petri dish before drying

W3 is the weight of sample+petri dish drying

Determination of pH

The pH of the samples was determined directly with a bench pH meter (Jenway 3,510, United Kingdom) after calibration using standard buffers 4.0 and 7.0 pH. Readings were carried out at room temperature and the average of triplicate determinations was recorded.

Data analysis

Laboratory findings were analyzed for accuracy. It was presented using Microsoft Excel 2019 and the Statistical Package for Social Sciences (SPSS) version 26 was used to analyze the data obtained. The data collected were subjected to one-way analysis of variance (ANOVA). A *p*-value of less than 0.05 was considered as significant at all points of analysis. The analysis was done using the mean fungal counts which were expressed in standard forms which were transformed into logarithmic values.

Ethical considerations

Ethical clearance was sought from the University of Health and Allied Sciences Research and Ethics Committee UHAS-REC A.9 [54] 20-21 prior to the conduct of the research. Findings obtained from this study will be for academic purposes and public health awareness creation only.

Results

Fungal counts

The mean fungal counts recorded on OGYEA for cowpea ranged from 1.83 to 2.6 log CFU/g. There was no statistically significant difference (p=0.660) among the cowpea vendors. For soybeans, it ranged from 2.22 to 2.74 log CFU/g. There was no statistically significant difference (p=0.788) among the soybean vendors. For brown beans, it ranged from 1.94 to 2.65 log CFU/g. There was no statistically significant difference (p=0.722) among the brown bean vendors. Again, for Bambara beans, it ranged from 2.29 to 2.45 log CFU/g for the different vendors. There was no statistically significant difference (p=0.977) among the Bambara bean vendors (Figure 1).

On SDA, the mean fungal counts recorded for cowpea ranged from 1.91 to 2.71 log CFU/g. For soybeans, it ranged from 2.22 to 2.67 log CFU/g while for brown beans, it ranged from 2.26 to 2.61 log CFU/g. Lastly, Bambara beans ranged from 2.52 to 2.84 log CFU/g for the different vendors. There was no statistically significant difference (p=0.564), (p=0.834), (p=0.886), and (p=0.906) among the cowpea, soybeans, brown beans, and Bambara beans vendors, respectively (Figure 2).

A total of 13 fungal species, namely; *Aspergillus niger*, *A. terreus*, *A. flavus*, *A. fumigatus*, *A. ochraceus*, *A. parasiticus*, *Fusarium oxysporum*, *Trichoderma harzianum*, *Rhizopus stolonifer*, *Penicillium digitatum*, *P. verucosum*, *Rhodotorula mucilaginosa*, and *Mucor racemosus* belonging to 7 genera namely: *Aspergillus*, *Fusarium*, *Trichoderma*, *Rhizopus*, *Penicillium*, *Rhodotorula*, and *Mucor* were isolated from cowpea, soybeans, brown beans, and Bambara beans (Figures 3 and 4; Table 3).

On cowpea, a total of 9 fungal species *A. niger*, *A. terreus*, *A. flavus*, *A. fumigatus*, *F. oxysporum*, *T. harzianum*, *R. stolonifer*, *P. digitatum*, *P. verucosum*, and *M. racemosus* belonging to 6 genera (*Aspergillus*,

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Fusarium, *Trichoderma*, *Rhizopus*, *Penicillium*, and *Mucor*) were isolated on both media. On soybeans, a total of 8 fungal species, *A. niger*, *A. fumigatus*, *A. ochraceus*, *R. stolonifer*, *F. oxysporum*, *M. racemosus*, *P. digitatum*, and *R. mucilaginosa* belonging to 6 genera (*Aspergillus*, *Rhizopus*, *Fusarium*, *Mucor*, *Penicillium*, and *Rhodotorula*) were isolated on both media. Again, on brown beans, a total of 7 fungal species, *A. niger*, *A. terreus*, *A. ochraceus*, *A. fumigatus*, *R. stolonifer*, *F. oxysporum*, and *M. racemosus* belonging to 4 genera (*Aspergillus*, *Rhizopus*, *Fusarium*, and *Mucor*) were isolated on both media. Finally, on Bambara beans, a total of 7 fungal species, *A. niger*, *A. fumigatus*, *A. flavus*, *A. parasiticus*, *F. oxysporum*, *R. stolonifera*, and *M. racemosus* belonging to 4 genera (*Aspergillus*, *Fusarium*, *Rhizopus*, and *Mucor*) were isolated on both media.

MC and pH

The average MC for the type of legumes from the different vendors was in the range of 7.64-8.43, 6.9-7.37, 7.53-12.15, and 6.74-8.33% for cowpea, soybean, brown beans, and Bambara beans, respectively (Figure 5). There were statistically significant difference (p<0.05) among cowpea and brown bean vendors.

pH values were in the range of 6.27±0.03, 6.53±0.02, respectively, for cowpea and soybean (Table 4). There was no statistically significant difference (p>0.05) among soybean and Bambara bean vendors (Table 3).

Table 1: The legume samples and corresponding interpretations

Code	Interpretation
CV1	Cowpea/vendor 1
CV2	Cowpea/vendor 2
CV3	Cowpea/vendor 3
SbV1	Soybean/vendor 1
SbV2	Soybean/vendor 2
SbV3	Soybean/vendor 3
BbV1	Brown beans/vendor 1
BbV2	Brown beans/vendor 2
BbV3	Brown beans/vendor 3
BBV1	Bambara beans/vendor 1
BBV2	Bambara beans/vendor 2
BBV3	Bambara beans/vendor 3

Table 2: Cultural and morphological characteristics of identified fungi

Fungal Specie	Cultural Characteristics	Morphological Characteristics
Mucor spp.	Large white colonies which turn into black later.	Erect sporangiophores are formed. Sporangiophore swell at the tip to form sporangia which are globular shaped. Columella is present.
Rhizopus spp.	White cottony mycelia with black dots and covers the entire plate.	Sporangiospores are produced inside a spherical sporangium. Columella is present on the top of the sporangiophore. Root-like rhizoids are found.
Penicillium spp.	Fast-growing colonies in green color with dense felt conidiophores.	Branched conidiophores with chains of conidia look like a brush.
Aspergillus spp.	Yellow to green, and black colonies with a distinct margin.	Conidiophores arise from a foot cell. Club-shaped vesicles on top of the conidiophores. Conidia are found in chains.
Fusarium spp.	White-pink sparse aerial mycelia becoming felty.	Macro conidia are sparse, borne on phialides with branched conidiophores (Septate banana-shaped).
Rhodotorula spp.	Soft, smooth, moist, and mucoid.	Round or oval-shaped budding cells.

Sources: (Da Cunha et al., 2013; Madrid et al., 2014; Samson et al., 1995; Samson et al., 2004; Samson and Van Reenen-Hoekstra, 1988)

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Table 3: Number of fungal species identified in cowpea, soybeans, brown beans, and Bambara beans from different vendors from 2 local markets in Ho Municipality (Ghana)

Fungi	Samples			
	Cowpea (n=9)	Soybeans (n=9)	Brown beans (n=9)	Bambara beans (n=9)
Aspergillus niger	+	+	+	+
Aspergillus flavus	+	-	-	+
Aspergillus fumigatus	+	+	+	+
Aspergillus ochraceus	-	+	+	-
Aspergillus parasiticus	-	-	-	+
Aspergillus terreus	+	-	+	-
Fusarium oxysporum	+	+	+	+
Mucor racemosus	+	+	+	+
Penicillium digitatum	+	+	-	-
Penicillium verucosum	-	-	-	-
Rhizopus stolonifer	+	+	+	+
Rhodotorula mucilaginosa	-	+	-	-
Trichoderma harzianum	+	-	-	-
Total	9	8	7	7

Table 4: pH values of cowpea, soybeans, brown beans, and Bambara beans from different vendors

Sample	pH	Mean±standard Deviation
CV1	6.38	6.39±0.03
	6.42	
	6.37	
CV2	6.24	6.3±0.05
	6.32	
	6.34	
CV3	6.3	6.27±0.03
	6.27	
	6.25	
SbV1	6.49	6.49±0.04
	6.52	
	6.45	
SbV2	6.56	6.53±0.02
	6.51	
	6.53	
SbV3	6.54	6.53±0.01
	6.52	
	6.53	
BbV1	6.31	6.29±0.02
	6.28	
	6.27	
BbV2	6.35	6.34±0.01
	6.33	
	6.34	
BbV3	6.49	6.45±0.03
	6.44	
	6.43	
BBV1	6.33	6.29±0.05
	6.31	
	6.24	
BBV2	6.34	6.32±0.03
	6.32	
	6.29	
BBV3	6.36	6.35±0.01
	6.34	
	6.35	

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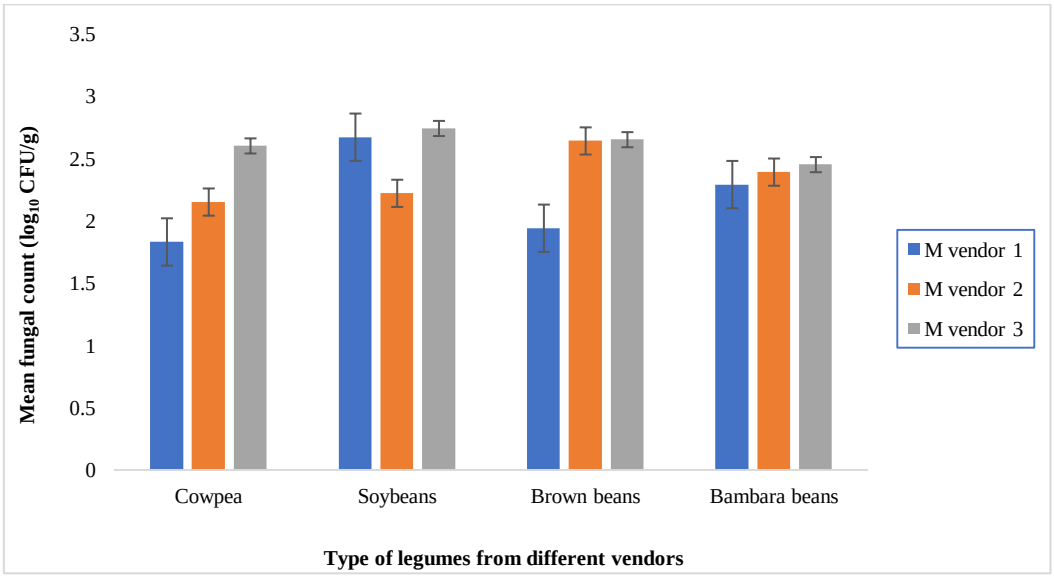

Figure 1: Mean fungal counts (log_{10} CFU/g) by each type of legumes from different vendors isolated from Oxytetracycline Glucose Yeast Extract Agar (OGYEA)

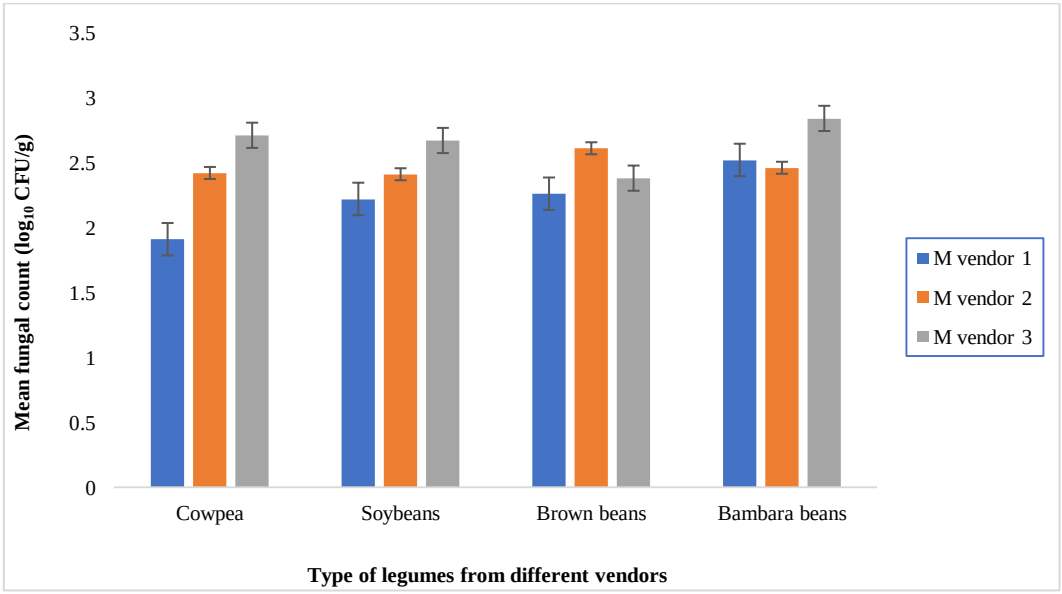

Figure 2: Mean fungal counts by each type of legumes from different vendors isolated with Sabouraud's Dextrose Agar (SDA)

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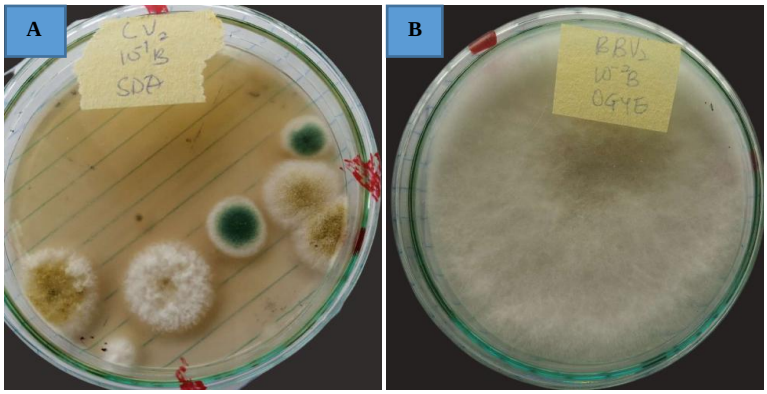

Figure 3: A: Showing the macroscopic view of *Aspergillus flavus, Fusarium oxysporum, Penicillium digitatum*; B: *F. oxysporum*

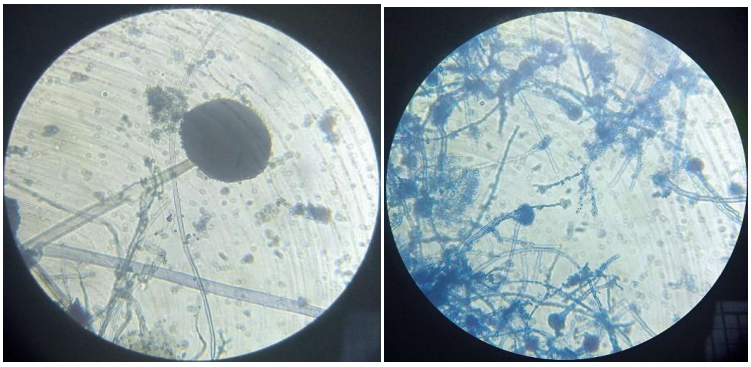

Figure 4: Microscopic view of *Aspergillus niger* and *Rhizopus stolonifer* from cowpea (Mg=×400)

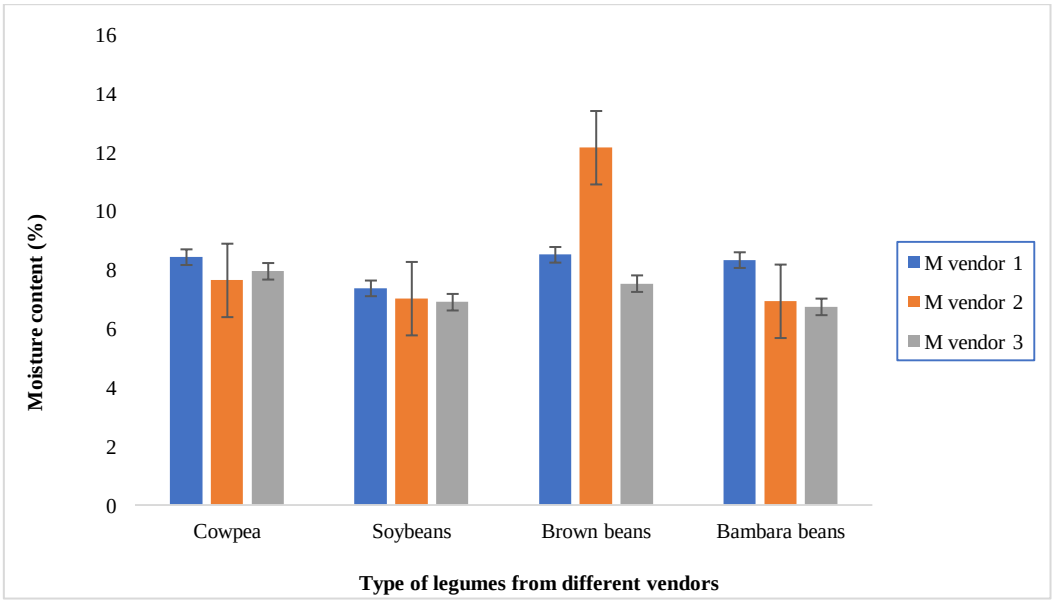

Figure 5: Average Moisture Content (MC) recorded on cowpea, soybeans, brown beans, and Bambara beans from different vendors

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Discussion

Molds contaminations of foods is affected by some environmental factors such as moisture, temperature, and pH. According to Yu and Ehrlich (2011), mold contamination is generally favored by the substrate's high activity, but other elements such as the substrate's type and temperature also play a role. From the results obtained in this work, the MC was averagely higher in brown beans compared to Bambara beans which was lower. Moisture is among several factors that facilitate the growth of molds on legumes. The high number of fungi can be attributed to open exposure of seeds to dust, high temperatures, and high humidity (Minamor and Appiagyei, 2017). The highest MC recorded in brown beans could be as a result of late harvesting and poor storage activities, which might have contributed to the numerous fungal species (Kortei et al., 2022). This observation is in line with a study by Popoola et al. (2010) who revealed that the high MC of 13-20% found in most stores in Maiduguri, Nigeria, is linked to the storage conditions there. High MC has been associated with a higher risk of fungal infection (Kortei et al., 2022). Nevertheless, molds can grow in low-moisture environments as well, which allows for growth seen in the other three legumes, especially Bambara beans. Foods having low water content have been shown to be contaminated with mold (Haddon and Schatzki, 2002). According to Chang et al. (1995), fungal growth on substrates including leather, wool, cotton, wood, etc. requires a minimum MC of 10%. The minimal MC for fungal development on the 4 legumes examined in our study was, however, much lower than 10%. Different substrate materials can have a big impact on the circumstances needed for fungal development, as evidenced by the variations in the minimum MC or equilibrium Relative Humidity (RH) needed for fungus growth in legumes. Table 3 demonstrates that the growth of various toxicogenic funguses was encouraged by all 4 legumes. Some of the basic ingredients most likely functioned as nutrients and promoted fungal development.

Results of the MC of Bambara beans obtained in this work agreed with the results of a study by Adjovi et al. (2019) who recorded a low MC level of 4.85 to 9.35% on Bambara groundnut from Benin. Generally, there is safe moisture for all grains, pulses, and legumes which can prolong their shelf life and preclude fungal invasion (Kortei et al., 2022). MC 10% or higher, after harvest of groundnut, predisposes the nuts to aflatoxin contamination (Chang et al., 1995). Therefore, timely drying and maintenance of safe moisture levels would achieve effective control of post-harvest mycotoxin contamination (Torres et al., 2014). Indeed, according to Peay and Bruns (2014), mold growth is generally curtailed when moisture is low and fungal spores are unable to have free water to commence de novo growth in storage.

The growth of fungi is influenced by a variety of factors, including moisture, pH, and temperature of the medium. The samples analyzed were slightly acidic (pH 6.27±0.03 and 6.53±0.02) as referenced by the pH scale. Additionally, a study by Kortei et al. (2021) found that "solom" samples of a regional beverage made from millet grew favorably on acidic (pH 3.03±0.09, 4.03±0.23) conditions. Again, a study by Agunbiade and Ojezele (2010) found that fortified breakfast cereals prepared from maize, sorghum, African yam beans, and soybeans, good fungal growth was observed at a little lower pH value of 4.88. This is consistent with Weyman-Kaczmarkowa and Pędziwilk (2000) published findings that fungus thrives in low pH environments with a pH of 4-6. On the other hand, a research by Yamanaka (2003) found that fungus thrives at a pH range of 7-9.

Fungal counts observed in this work are similar to that reported by Popoola et al. (2010) in Maiduguri, Nigeria where the total fungal counts recorded were higher in peanuts 1.5×10^3 CFU/g (3.18 log CFU/g) than cowpea 1.2×10^3 CFU/g (3.08 log CFU/g). Recently, in a related study, Kortei et al. (2022) reported fungal population counts ranged from 2.01 to 2.16 log CFU/g samples with a final 6 month count of 1.67-2.60 log CFU/g in groundnuts sampled from the Volta region of Ghana. Improper farming methods and poor handling of products from the farm to the market could account for the wide range of fungal counts encountered in this study.

The findings of this study indicated that 13 fungal species were associated with cowpea, soybean, brown beans, and Bambara beans. The presence of these 13 fungal species could be due to poor handling and poor storage conditions and this could lead to harmful effects if precautionary measures are not taken. In agreement with the results obtained in this study, Minamor and Appiagyei (2017) isolated a total of 4 fungal genera, namely; *Aspergillus*, *Fusarium*, *Penicillium*, and *Mucor* on maize, cowpea, groundnuts, and Bambara beans purchased from 2 popular local markets and 2 supermarkets in the Accra metropolis, Ghana. A related study by Ogundipe et al. (2019) confirmed the presence of *Aspergillus*, *Penicillium*, and *Fusarium* genera on Bambara groundnut from Nigeria, which is in line with the results obtained in this study. Similarly, Sahab et al. (2016) isolated *A. flavus*, *A. terreus*, and *A. niger* from cowpea and soybeans from Egypt. In this study, the findings of fungal species isolated from the legume samples is in conformity with Olagunju (2019) who reported that different fungal species such as *A. flavus*, *A. niger*, *A. tamarii*, *P.*

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citrinum, and *P. oxalicum* were discovered in Bambara groundnut samples obtained from retail stores and open markets in Durban, South Africa. Again, a study by Fagbohun and Faleye (2012) isolated *A. niger*, *A. flavus*, *Rhizopus* spp., *Mucor* spp., and *A. fumigatus* in sundried groundnut from Nigeria which is similar to the results obtained in this study. In a related study, Adjovi et al. (2019) reported contamination of Bambara groundnut by *Mucor, Rhizopus, Alternaria,* and *Aspergillus* from Cotonou's main market in Benin. Again from Benin, Houssou et al. (2009) reported a wider scope of fungi which included; *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., *Chaetomium* spp., *Chrysonilia* spp., *Cladosporium* spp., *Monascus* spp., *Phoma* spp., *Neosartorya* spp., *Rhizopus* spp., and *Mucor* spp. from cowpea and were within the same range of fungi observed in this study. This observation could be attributed to perhaps the similar prevailing favorable tropical weather conditions which allow most fungi to flourish.

From Egypt, Embaby and Abdel-Galil (2006) isolated seed-borne fungi which contaminated some legume seeds (bean, cowpea, and lupine) and bore 200 fungal isolates belonging to 5 genera namely *Alternaria, Aspergillus, Epicoccum, Fusarium,* and *Trichoderma*. Saleem and Ebrahim (2014) reported most common genera of fungi found on legume seeds obtained from Saudi Arabia, including *Alternaria, Aspergillus*, *Emericella, Mucor, Mycosphaerella*, *Penicillium,* and *Rhizopus*. Presumably, the different miscellany of fungi colonized these legumes due to the different climatic conditions in that geographic location.

Food products that have high levels of mold growth during processing stages such as harvesting, drying, and storage, which may be contaminated with a mycotoxin (Ozturkoglu-Budak, 2016). A study by Henning (2005) reported that *Phomopsis* sp., *F. semitectum, Sclerotinia sclerotiorum, S. rolfsii,* and *A. flavus* that can cause germination problems and mycotoxin accumulation are the most commonly seed-transmitted pathogens that attack soybean. According to Mzungu et al. (2018), molds of the genera *Aspergillus, Mucor,* and *Rhizopus* heavily contaminated with maize, millet, guinea corn, groundnut, and groundnut cake in Nigeria. Many of the fungi are well-known pathogens that cause seed decay, root rot, stem cankers, wilting, necrosis, and/or death of infected bean plants; for example, *Fusarium* spp., *Penicillium* spp., and *A. flavus* are the predominant species detected in common bean in Cuba (De La Parte et al., 2014). Generally, the preponderant species of fungi identified among legumes belong to the genera *Aspergillus, Fusarium, Mucor, Rhizopus,* and *Penicillium* (Adjovi et al., 2019; Embaby et al., 2013; Minamor and Appiagyei, 2017; Saleem and Ebrahim, 2014). This is in accordance with the results obtained in this work where molds of the genera *Aspergillus, Mucor, and Rhizopus* were identified on cowpea, soybeans, brown beans, and Bambara beans. Contrary to the results of this study except for *F. oxysporum*, Edema (1995) stated that *Colletotrichum, Ascochyta phaseolorum, Cladosporium vignae, F. solani, Tracheiphilum lindemuthianum,* and *F. oxysporum* were the most common fungi that infected cowpea in Uganda. Again, many of the seed-borne pathogenic fungi detected in 'INTA Rojo' (a cultivar of bean) as reported by Marcenaro and Valkonen (2016) were previously infrequent and unreported in Nicaragua, and reports on the occurrence of some, such as *F. incarnatum, Lasiodiplodia theobromae, Corynespora cassiicola,* and *Diaporthe*, as seed-borne pathogens of common bean are rare elsewhere.

In Argentina, Zelaya et al. (2013) reported that all fungi isolated were mitosporic fungi and belonged to Ascomycota. The most common fungi identified included species that belong to *Alternaria, Fusarium, Sclerotinia, Phomopsis, Rhizoctonia,* and *Cladosporium* genera. Afolabi et al. (2020) reported that moulds belonging to *Aspergillus, Fusarium,* and *Penicillium* were recovered from 99% of the samples. In both cowpea varieties, *Aspergillus* (52-53%) dominated *Fusarium* (29-30%), and *Penicillium* (17-20%) in 2 cowpea varieties collected from various markets in southwestern Nigeria.

The most prevalent pollutants were *Cladosporium* spp., *Alternaria* spp., *Aspergillus* spp., *Rhizopus* spp., and *Penicillium* spp. in a research by Domijan et al. (2005) however, the frequency of positive samples was not uniform across Croatia. Essentially, the species present at harvest may diminish or become overgrown with time while the "storage" fungus, including species like *P. verrucosum* might increase fast. As a result, the makeup of the fungal population after harvest will depend on the length and circumstances of storage and/or may compel other fungal species to diminish.

Kortei et al. (2022) explained that antibiosis, which is a biological interaction between two or more organisms, can also be an antagonistic association between an organism and the metabolite substance produced by another. This phenomenon of antibiosis is common among fungal species in a closed ecological niche and may be responsible for the phenology of the fungal species resident in the legumes. Seed-borne pathogenic fungi in legumes used for seeds decrease germination, expansion, growth, and yield, whereas in legumes used for food they can reduce the nutritional value or produce toxins making the legume unfitted for consumption. Food or feed products from these legumes are often demoted if they are contaminated with fungi and consequently mycotoxins of health and pathological importance (Jolly et al., 2009).

Mycotoxins are natural toxic compounds produced by fungal species (Hathout et al., 2020; Hathout and Aly,

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2014; Kortei et al., 2021; Reddy et al., 2010). They cause health hazards and even death in humans and animals when they occur in large quantities in food. It is worthy to note that regarding the mycotoxins produced by toxicogenic species, no amount of toxin above the zero level is regarded as safe. "Reduction to As Low As Reasonably Achievable" is the endorsement of Joint Experts Committee on Food Additives (JECFA) concerning the safe level in foods following the significant genotoxic, carcinogenic, etc. probabilities of these toxins (Hathout et al., 2020).

In the present study, the results revealed a possible natural occurrence of diverse types of mycotoxins in the legume samples. *A. flavus*, *A. niger*, *A. parasiticus*, *A. fumigatus*, *A. terreus*, and *A.ochraceaus* isolated from these legumes in this work indicated a possible contamination of aflatoxins (produced chiefly by *A. parasiticus* and *A. flavus*) which is categorized as a class 1 carcinogen (World Health Organization and International Agency for Research on Cancer, 1993). Ingested even at the tiniest quantities via the skin, aflatoxins have teratogenic, carcinogenic, hepatotoxic, and mutagenic outcomes on human health (Pleadin et al., 2019; Zain, 2011), due to their accumulative potentials. Malnutrition, notably "Kwashiorkor" has been reported to be positively correlated with aflatoxins by several researchers (Achaglinkame et al., 2017; Onyemelukwe et al., 2012; Oyelami et al., 1997; Soriano et al., 2020) in Africa. Prolonged intake of foods contaminated with aflatoxins leads to severe and unimaginable conditions listed herein. When food contaminated with aflatoxins is ingested, the aflatoxins are then transformed into aflatoxin-8,9-epoxide metabolite in the liver, which has been implicated in numerous hazardous consequences in the body (Bbosa et al., 2013; Eaton and Gallagher, 1994; Kew, 2013). Epidemiological studies of human populations exposed to diets naturally contaminated with aflatoxins revealed a relationship between the high incidence of liver cancer in Africa and elsewhere and dietary intake of aflatoxins (Wild and Montesano, 2009). For people who are disease-ridden with hepatitis B and C, which is common in Sub-Saharan Africa, aflatoxin ingestion surges the danger of liver cancer by exceeding ten-fold compared to either exposure unaccompanied (Turner et al., 2003).

Fumonisins are a possible human carcinogens (World Health Organization and International Agency for Research on Cancer, 1993) produced by *Fusarium* species which have been implicated in a number of animal diseases such as leuco-encephalomalacia in equines, which involves a massive liquefaction of the cerebral hemisphere of the brain with neurological manifestations such as abnormal movement, aimless circling, lameness; porcine pulmonary oedema; rat liver cancer; and haemorrhage in the brain of rabbits (Rheeder et al., 2002). It can cause hepatotoxicity and nephrotoxicity in many animals (He et al., 2002). At greater quantities of contact, fumonisin B_1 has been shown to produce liver cancer, decreased the life span in female mice, and induced liver carcinoma in male rat (Wagacha and Muthomi, 2008). Significant positive correlations between fumonisin and aflatoxin levels in different foods have been reported globally (Castells et al., 2008; Manjula et al., 2009; Taye et al., 2016).

Zearalenone (previously known as F-2) is also produced by *F. graminearum* and related species, principally in wheat and maize but also in sorghum, barley, and compounded feed. Zearalenone and its derivatives produce estrogenic effects in various animal species (infertility, vulval oedema, vaginal prolapse, and mammary hypertrophy in females and feminization of male atrophy of testes and enlargement of mammary glands) (Peraica et al., 1999).

Trichothecenes are mycotoxins produced mostly by members of the *Fusarium* genus, although other genera such as *Trichoderma*, *Trichothecium*, *Myrothecium*, and *Stachybotrys* are also known to produce these compounds. To date, 148 trichothecenes have been isolated, but only a few have been found to contaminate food and feed. In several cases, trichothecene mycotoxicosis was caused by a single ingestion of bread containing toxic flour or rice (Bhat et al., 1989; Wang et al., 1993). In experimental animals, trichothecenes are 40 times more toxic when inhaled than when given orally (Peraica et al., 1999).

Conclusion

The presence of some mycotoxigenic fungi in legumes examined in this study showed the potential health hazards in the local people of Ho, Ghana. Our findings also indicated that legumes grown in the study area were contaminated by a broader variety of mycotoxigenic fungus. This suggests that mycotoxin analysis needs to be expanded to include potential mycotoxins including fumagillin, ochratoxin A, and also fumonisin, which have been shown to have negative effects on human health.

Author contributions

N.K.K. and P.A. designed the original study, did the experiments, and wrote the manuscript; P.A., T.A., and N.K.K. were responsible for statistical analysis; N.K.K., T.A., and P.A. interpret the experiments and revised the manuscript; P.A., N.K.K., and T.A. did the sampling. All authors read and approved the final manuscript.

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Conflicts of interest

The authors have no conflicts of interest to disclose in this study.

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