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# Mycoflora and influence of moisture on the mycological profile and their mycotoxigenic potential in some imported spices and seasonings on the Ghanaian market

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**Authors' Contribution**: **Addo, J.** analyzed the data, **G. T. Odamtten** identified the fungal species and **G. Anyebuno** performed the mycotoxin analysis.

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## ABSTRACT

The liberalized Ghanaian economy has resulted in the influx of many internationally accepted and shoddy goods into the country. This has spurred on the study of some four of such spices, (seasoned meat tenderizer, poultry seasoning, ground cinnamon and sausage spices) with the view to ascertaining their mycological qualities when expose to different environmental relative humidity (ERH) and mycotoxin contamination using the conventional methods. The samples absorbed moisture differently at 75%, 85% and 95% ERHs. There was commensurate increase in resident mycoflora population of nearly 2 log cycles as the incubation ERH increase from 75%-95%. Each sample recorded 10 fungal genera, with 6 common groups (*Absidia, Aspergillus, Eurotium, Mucor, Penicillium* and *Rhizopus*). Fungal species diversity encountered on all samples can be ranked as follows: seasoned meat tenderizer (24 species), > sausage spices (19 species), > ground cinnamon (18 species) > poultry seasonings (17 species). Variable vegetative and colony growth of three dominant *Aspergillus* (*A. niger, A. flavus, A. fumigatus*) species on spice-based media, indicated they could serve as suitable substrates for growth and mycotoxins formation. Of all spices tested only sausage spices contained aflatoxin B1 (1.951 µg/kg), aflatoxin B2 (0.552 µg/kg) and ochratoxin A (26.470 µg/kg). Results obtained underscores need for continuous use of existing regulatory specifications to monitor quality of imported and locally manufactured dehydrated foods and spices to protect the populace from consuming products laden with mycoflora and mycotoxins detrimental to their health.

**Keywords**: Mycoflora, aflatoxin, ochratoxin A, Environmental Relative Humidity; Isotherms.

**INTRODUCTION:** Spices are an important group of agricultural commodities with the ability to influence the aroma, taste, flavor, color, pungency and nutritional values of food (Singh *et al.*, 2004). Some spices possess medicinal and aromatherapeutic properties which have made them very relevant raw materials in the pharmaceutical, perfume and cosmetic industries. In the past, when food preservation methods were inadequate, spices were used as preservatives to prevent spoilage of food products. Reliance on convenience foods, bakery products and processed foods (soup mixes, prepared sauces meat, fish, and vegetable) have increased the consumption of spices and seasonings. Since all the spices and seasonings are from plants sources, they have been generally recognized as safe (GRAS), hence their inclusion into food products is to control food-borne pathogens and improve its quality. However, spices and seasonings are also recognized as important vehicles for microbial contamination since most of these commodities are grown, harvested, threshed, processed, transported and stored using traditional or local methods with no or little regard for microbial safety protocols (Hashem and Alamri, 2010). Depending on the source, preparation and packaging, the microbiome of spices and seasonings may include spore-forming bacteria, spoilage bacteria and fungi. Dried material from plant origin and spices are commonly known to be heavily contaminated with xerophilic storage moulds and bacteria (Romagnoli *et al.*, 2007). Unless spices and food seasoning are decontaminated, they may change quality of the nutritional menu items and cause foodborne illnesses, food spoilage and intoxication (Bakobie *et al.*, 2017) by the introduction of mycotoxins such as aflatoxins, ochratoxins, *Fusarium* toxins (sterigmatocystin, fumonisins, T2 Toxins etc), patulin (Ahene *et al.*, 2011). By far the most important phenomenon of public health importance in storage fungi, is the formation of mycotoxins. Mycotoxins are secondary metabolites produced by microscopic fungi that are capable of causing disease and death in humans and other animals at low concentrations (Bennett and Inamdar, 2015). The most common fungal species that produce mycotoxins belong to the genera *Aspergillus, Penicillium, and Fusarium*. Some species of these genera have potential to produce different mycotoxins such as aflatoxins, ochratoxin, and citrinin. Mycotoxins are usually defined by the producing organism. For instance, aflatoxins are from some species of *Aspergillus* (*A. flavus, A. parasiticus, A. nomius* etc.) and are recognized as genotoxic and carcinogenic (Jeswal and Kumar, 2015). *A. flavus* strains produces aflatoxins B1 and B2, G1 and G2 while *A. parasiticus* and *A. nomius* also produce aflatoxins G1 and G2. Aflatoxins M1 and M2 (which are the hydroxylated aflatoxins B1 and B2 respectively) are found in milk or milk products obtained from livestock which have fed on contaminated feed silage. *Aspergillus alutaceus* (formally *A. ochraceus*) are associated with ochratoxin A which is nephrotoxic. Nonetheless, it is sometimes believed that fungal species purported to make mycotoxins are based on an erroneous association with a particular species (Frisvad *et al.*, 2006). In various spices, many potential toxins producing fungal species such as *Apergillus flavus* (aflatoxins), *A. fumigatus*(fumigillin), *A. niger* (nigerone), *A. alutaceus*

(ochratoxin), *Fusarium verticillioides* (fumonisins), *Penicillium expansum, P. digitalium* (patulin) and *Trichoderma viride* (Trichodermin) were encountered. An excessively high storage humidity can promote the development of filamentous fungal flora in spices that can lead to their deterioration before their stipulated expiry dates. Fungi have minimum water activity ($a_w$), (an indication of available free water for growth of microorganism) for growth and toxin formation above which the food is susceptible to contamination with microbial toxin. Water activity ($a_w$) effectively quantified the relationship between moisture in foods and the ability of microorganisms to grow on them (Pitt and Hocking, 2009). Opening the seal of spices and seasonings packages is likely to infract the integrity of the ideal conditions for extension of shelf-life and so even under refrigeration; the product may be predisposed to contamination (Odamtten *et al.*, 2018).

**OBJECTIVES:** The objectives of the current study were: to assess the mycological quality and the influence of moisture (ERH) on the mycological profile of four imported spices and seasoning, namely, seasoned meat tenderizer, poultry seasoning, ground cinnamon and sausage spices; to ascertain the presence of potential mycotoxigenic species in the selected samples; to determine whether Aflatoxin and Ochratoxin are formed in the samples during storage; and to confirm that the substrate (spices and seasonings) serve as a source of nutrient for the resident mycoflora.

**MATERIALS AND METHODS:** **Source of spice and seasoning samples:** The four spices and seasoning were collected from the Marina Mall (5.60279° N, 0.17611 ° W) located at Airport City Accra in the Greater Accra Region of Ghana. Seasoned meat tenderizer (salt, dextrose onion, annatto, paprika, garlic, calcium stearate and papain), poultry seasoning (salt, ground herbs and spices, ground mustard, soybean oil), ground cinnamon (71 g) were packaged in plastic containers and sausage spices (coriander, allspice, black pepper, cinnamon, clove, nutmeg, ginger, cumin. Manufactured by Gardenia) packaged in polyvinyl sachets.

**Quantitative estimation of mycoflora population:** The mycoflora population in the spices and seasonings were determined by the Decimal Serial Dilution technique following the method described by ISO (1999). About 1g of each sample was dissolved in 100 mL of 0.1% peptone water and was shaken at 140 RPM for 10 min on an orbital shaker (Gallenkamp, England). Serial dilutions up to $1:10^4$ were prepared and 1 mL aliquots from each dilution level were plated on 20 mL of Oxytetracycline Glucose Yeast Extract agar (OGYE, Oxoid CM0545), Dichloran Rose Bengal Chloramphenicol agar (DRBC, Oxoid CM0727) and Potato Dextrose Agar (CM0139). The plates were incubated at 30 ± 2°C for 7 days. Colonies of mycoflora that appeared after 7 days of incubation were counted and calculated as $\log_{10}$ CFU/g sample.

**Isolation and identification of mycoflora:** Fungal colonies which appeared after the prescribed incubation were isolated and identified by their cultural and color morphological characteristics (microscopic and macroscopic) as described in Standard Identification Manuals by Barnett and Hunter (1972).

**Moisture sorption isotherms of the selected spices and seasonings:** Moisture sorption isotherms of the selected spices and seasonings were determined by the static desiccator method (humidity chambers) using mixture solutions of standard glycerol: water (to make solution total of 300 ml) which provided the humidity (75%, 85% and 95% ERH). The glycerol: water mixtures were prepared in the following combinations: 75%- (175: 125) ml, 85%- (135: 165) ml and 95%- (96: 204) ml. Exactly 10 g of each of the four samples (Seasoned meat tenderizer, Poultry seasoning Ground cinnamon and Sausage spices) in duplicates were placed in open petri dishes and exposed in the humidity chambers (desiccators) and allowed to absorb moisture for 8 days. The percentage change in weight were determined and recorded after 8 days. Method of preparing solutions of glycerol-water were modified after that of Braun and Braun (1958).

**Effect of environmental relative humidity (ERH) on growth of resident fungi:** At the end of the incubation period of 8 days, decimal serial dilution up to $1:10^3$ were prepared (followed the above-described procedure). Colonies of mycoflora that appeared after 7 days of incubation were counted and calculated as $\log_{10}$ CFU/g sample and the fungal species were isolated and identified. Here, $\log_{10}$ CFU/g data obtained on the three media (OGYE, DRBC and PDA) served as replicates.

**Influence of spices and seasoning-based media on vegetative growth of selected resident**: Exactly 5.0 g of the spice/seasoning powder was suspended in 250 mL of distilled water and the mixture stirred gently to dissolved completely. About 30 mL of each medium (seasoned meat tenderizer broth, poultry seasoning broth, ground cinnamon broth and sausage spices broth) was then decanted in conical flasks in triplicates. The mixture was then sterilised at 121°C for 45 mins. Three millimeters (3 mm) discs of the selected resident fungi from pure cultures (*A. niger, A. flavus, and A. fumigatus*) were inoculated into the broth media in conical flasks and onto solid agar medium on agar plate and all cultures incubated at 30 ± 2 °C for 7 days. Growth in the liquid medium was assessed using the conventional Mycelium Dry Weight Method and those of solid agar medium assessed by Radial Colony Diameter Method modified after those of Taniwaki *et al.* (2006) and Paster *et al.* (1983). The dry weight of the mycelium was recorded and calculated as (Mean dry weight of mycelium ± S.E.) mg and colony diameter as (Mean colony diameter ± S.E.) mm.

**Mycotoxin analyses of samples:** The aflatoxins (produced by *A. flavus*) and ochratoxin A (produced by *A. alutaceus*) levels in the samples were determined using the high-pressure liquid chromatography HPLC (Model Agilest 1260 Series, USA) at the Food Research Institute CSIR, Ghana following the methods outlined by Pons (1979). About 50 g each of the four spices and seasonings sample were spiked with $2.5x10^3$ spores/µL of *A. flavus* or *A. alutaceus* and incubated at 100% ERH for 7 days (Odamtten *et al.*, 2018). The samples were subjected to Aflatoxin B1, B2, G1 and G2 and total aflatoxin and Ochratoxin A analysis following the procedure of Odamtten *et al.* (2018).

**Statistical analysis:** Numerical data obtained were subjected to ANOVA where appropriate using the Genstat Statistical Package (12th Edition) to evaluate data at $p<0.05$ level of significance. Difference between means were separated by Duncan's Multiple Range Test at 5 % Least significant difference (LSD).

**RESULTS AND DISCUSSION:** The Food and Drugs Authority (FDA) and the Ghana Standards Authority (GSA) are the principal agencies responsible for checking the quality of food and pharmaceutical products on the Ghanaian market. Even though, ISO Standardization methods, Good Manufacturing Process,

GMP, Hazard Analysis at Critical Control Point, HAACP and International Commission on Microbiological, ICMSF protocols for microbial safety measures are presumed to be adhered to at both manufacturing and importation points, the survival of ubiquitous microbial species in dehydrated food products in a well-known phenomenon (Odamtten *et al.*, 2018).

**Influence of Environmental Relative Humidity on Moisture Sorption by the four imported Spices and Seasonings**: The dehydrated selected spices and seasonings previously packaged in the various air-tight and moisture-resistant containers when exposed to different ERHs of 75%, 85% and 95%, absorbed moisture at different rates. It was presumed that, 75% ERH would be close to the environment of the containers of the various spices and seasonings but 85% and 95% ERHs would provide moisture for absorption by the spices and seasonings. Generally, the moisture contents increased as the humidity level increased. The moisture sorption of the seasoned meat tenderizer was generally the highest compared to the other spices and seasonings (figure 1). At 95% ERH, the seasoned meat tenderizer absorbed about 96.5% of moisture of its weight and 54.6% and 31.5% at 85% ERH and 75% ERH respectively (figure 1). The seasoned meat tenderizer became deliquescent at both 85% and 95% ERHs. This was followed by the poultry seasoning, absorbing moisture of 49.5%, 30.9% and 10.2% at ERH's of 95%, 85% and 75% respectively and the sausage spices absorbing moisture of 6.8% ,6.3% and 4.7% of their weights at 95%, 85% and 75% ERHs respectively. The ground cinnamon absorbed the least moisture of 3.4%, 2.7%, and 1.7% of its weight at 95%, 85% and 75% ERHs respectively. The moisture absorption by the seasoned meat tenderizer, poultry seasoning, ground cinnamon and sausage spices were different at 75-95% ERH. This implies the samples were hygroscopic but varied in hygroscopicity, ie ground cinnamon absorbed the least moisture at all ERHs studied, followed by sausage spices, poultry seasoning, seasoned meat tenderizer in increasing order (figure 1). This was however not surprising since all the ingredients of the ground cinnamon (cinnamon) and sausage spices (coriander, allspice, black pepper, cinnamon, clove, nutmeg, ginger, cumin) are considered by Voelker and Sommer (2020) as low/medium moisture sorption spices/seasonings and that of seasoned meat tenderizer (salt, dextrose onion, paprika, garlic, papain) as medium/high moisture sorption seasonings. The low moisture sorption behavior of the ground cinnamon spice may be probably due to its less porous particle, fine nature, as observed by Voelker and Sommer (2020). Odamtten *et al.* (2018) reported the hygroscopic nature and deliquescence of some seasonings at 55, 75, 85, 95% ERH, and stated that the imported hermetically sealed seasonings are normally not manufactured to withstand the usual vicissitude of the changing fortunes of the tropical weather conditions when opened and dissolved quickly in thin air.

**Comparative effect of ERH on the mycoflora profile of resident fungi:**

**Mycoflora population:** The population of resident mycoflora in the spices and seasonings was significantly influenced by the prevailing environmental conditions (figure 2). Mycoflora population significantly varied from the initial through to 95% ERH on seasoned meat tenderizer from 2.57- 4.46 $\log_{10}$ CFU/g sample; poultry seasoning, 2.34-3.79 $\log_{10}$ CFU/g sample; ground cinnamon, 2.79 - 4.06 $\log_{10}$ CFU/g sample and the sausage spices 2.61- 4.67 $\log_{10}$ CFU/g sample (figure 2). The differences in mycofloral population in the initial sample through to 95% ERH varied by more than 1 log cycle. There was a commensurate increase in resident mycoflora population by about 0.5 log cycles at 85% ERH and 95% ERH for ground cinnamon, sausage spices, except for Seasoned meat tenderizer, and poultry seasoning (figure 2). The initial resident mycoflora population in the samples varied according to the media employed in the isolation of the fungal species. There was commensurate increase in resident mycoflora population by a near 2 log cycles as incubating ERH increased from 85-95% ERH (figure 2). This is excepted because increasing the ERH make more free-water available for microbial growth. This observed relationship between the higher ERHs and concomitant increase in mycoflora population has also been reported by Pitt and Hocking (2009), who reported that there exists a relationship between moisture in foods and the ability of microorganisms to grow. Most food with water activity above 0.95 $a_w$ (95% ERH) will provide enough free moisture to support the growth of spoilage microbes and can affect the safety and quality of foods. This finding agrees with the report of Odamtten *et al.* (2018), that exposing the spices/seasonings to the environment as a result of improper sealing of the package after opening, may result in the increase of the moisture content and as a springboard for the opportunistic microorganisms to commence *de novo* growth even under cold storage conditions.

**Mycoflora profile of resident fungi in samples:** About 35 fungal species from 14 genera (*Absidia, Acremonium, Aspergillus, Botrytis, Nigrospora, Cladosporium, Eurotium, Fusarium, Mucor, Paeciliomyces, Penicillium, Rhizopus, Saccharomyces, Syncephalastrum*) were isolated from the spice and seasoning on 3 media (table 1). Each sample recorded 10 genera, with 6 common groups (*Absidia, Aspergillus, Eurotium, Mucor, Penicillium, Rhizopus*). Fungal species diversity encountered on all the four spice and seasoning samples can be rank as follows: Seasoned meat tenderizer (24 species), > sausage spices (19 species), > Ground cinnamon (18 species) > poultry seasonings (17 species) (table 1). *Aspergillus* species (*A. alutaceus, A. flavus, A. fumigatus, A. niger, A. oryzae, A. striatus, A. tamarii, A. terreus, A. sulphureus, and A. wentii*) predominated over the others followed by *Penicillium* (*P. aurantiogriseum, P. brevicompactum, P. camemberti, P. charlesii, P. digitatum, P. expansum, P. glabrum, P. italicum, P. verrucosum*), members of the Mucorales (*Absidia* (*A. corymbifera, A. glauca, Mucor hiemalis, Rhizopus* (*R. oryzae, R. oligosporus, R. stolonifer*), *Syncephalastrum racemosum*), and *Fusarium* (*F. chlamydosporum* and *F. poae*) (table 1). Seasoned meat tenderizer and sausage spices recorded single species of *Cladosporium herbarum* and *Eurotium amstelodami*. *Botrytis cinerea*, *Nigrospora oryzae* were peculiar species on poultry seasoning and *Paecilomyces variotii* was only isolated from ground cinnamon (table 1). Interestingly, the four spices shared almost the same contaminating members of the Mucorales (*Absidia glauca, Mucor hiemalis, Rhizopus stolonifer, R. oligosporus, R. oryzae, Syncephalastrum racemosum*) and the potential mycotoxin-producing species (*Aspergillus flavus*: aflatoxin; *A. alutaceus*: ochratoxin A; *A. fumigatus*: Fumigallin; *A. sulphureus*: ochratoxin A; *Penicillium* species: patulin) (table 3). As expected, exposure to the various ERHs increased the overall fungal diversity of the samples from 24 species in the initial (75% ERH) samples to 28 and 27 species at 85% ERH and 95%

| S. no | Fungal species | SMT | PS | GC | SS |
|---|---|---|---|---|---|
| 1. | *Absidia corymbifera* (Conh) Sacc. & Trotter [3] | + | - | - | - |
| 2. | *Absidia glauca* Hagem [1, 2, 3] | - | + | + | + |
| 3. | *Acremonium strictum* W.Gams [1] | - | + | + | + |
| 4. | *Aspergillus alutaceus* Berk & M.A. Curtis [1] | + | - | + | + |
| 5. | *A. flavus* Link [1, 2, 3] | + | + | + | + |
| 6. | *A. fumigatus* Fresen. [1, 2, 3] | + | + | + | + |
| 7. | *A. niger* Van Tieghem [1, 2, 3] | + | + | + | + |
| 8. | *A. oryzae* (Ahlburg) Cohn [1, 2, 3] | + | - | - | + |
| 9. | *A. striatus* J.N.Rai, J.P. Tewari &Mukerji [1] | + | - | - | - |
| 10. | *A. sulphureus* (Fresen.) Wehmer [1, 2, 3] | | + | | + |
| 11. | *A. tamarii* Kita [1] | + | - | + | - |
| 12. | *A. terreus* Thom [1, 2, 3] | + | + | + | - |
| 13. | *A. wentii* Wehmer [1] | - | + | - | - |
| 14. | *Botrytis cinerea* Pers. [2] | - | + | - | - |
| 15. | *Cladosporium herbarum* (Pers.) Link [1, 2, 3] | + | - | - | + |
| 16. | *Eurotium amstelodami* L. Mangin [1,2,3] | + | + | + | + |
| 17. | *F. chlamydosporum* Wollenw. & Reinking [3] | + | - | - | - |
| 18. | *F. poae* (Peck) Wollenw. [1, 2, 3] | + | - | + | + |
| 19. | *Mucor hiemalis* Wehmer [1, 2, 3] | + | + | + | + |
| 20. | *Nigrospora oryzae* (Berk. & Broome) Petch [1] | - | + | - | - |
| 21. | *Paecilomyces variotii* Bainier [2] | - | - | + | - |
| 22. | *Penicillium aurantiogriseum* Dierckx [1] | + | - | - | + |
| 23. | *P. brevicompactum* Dierckx [1] | + | - | - | - |
| 24. | *P. camemberti* Thom [1] | + | + | - | - |
| 25. | *P. charlesii* Smith, G. [2, 3] | - | - | + | - |
| 26. | *P. digitatum* (Pers.) Sacc. [2] | - | + | - | + |
| 27. | *P. expansum* Link [1, 2, 3] | + | + | + | + |
| 28. | *P. glabrum* (Wehmer) Westling [2, 3] | - | - | + | + |
| 29. | *P. italicum* Wehmer [2, 3] | + | - | + | - |
| 30. | *P. verrucosum* Dierckx [2] | + | - | - | - |
| 31. | *Rhizopus oligosporus* Saito [1] | - | - | - | + |
| 32. | *R. oryzae* Went & Prins. Geerl. [1, 2, 3] | + | + | + | + |
| 33. | *R. stolonifer* (Ehrenb.) Vuill. [3] | + | - | - | - |
| 34. | *Saccharomyces cerevisiae* Meyen ex E.C. Hansen [1, 2, 3] | + | - | + | - |
| 35. | *Syncephalastrum racemosum* J. Schröt. [1, 2, 3] | + | + | - | + |
| Total | Species | 24 | 17 | 18 | 19 |
| | Genera | 10 | 10 | 10 | 10 |

Table 1: Total list of the initial mycoflora resident in the four spices and seasonings on three mycological media for 7 days at 30 ± 2°C. DRBC; 2=PDA; 3= OGYE; +: Present; -: Absent, SMT: Seasoned meat tenderizer; PS: Poultry seasoning; GC: Ground cinnamon; SS: Sausage spices.

ERH respectively (table 2). The occurrence of some of the resident fungal species changed after the samples were stored at the test ERHs. For instances, *A. wentii*, *B. cinerea, N. oryzae* and *Paecilomyces variotii* were not isolated when the samples were stored at 85% ERH and 95% ERH. Also, *Penicillium verrucosum* and *Fusarium chlamydosporum* were only present at 85% and 95% ERH respectively and finally *Penicillium aurantiogriseum, P. charlesii, P. glabrum, P. italicum* were encountered when the ERHs were between 85% -95%. In all instances, *Aspergillus* species > *Penicillium* species > *Mucoralean* species. Clearly, eighteen (18) fungal species persisted throughout at initial (75%), 85% and 95% ERHs, namely, *Absidia corymbifera, A. glauca, Aspergillus flavus, A. fumigatus, A. niger, A. oryzae, A. tamarii, Eurotium amstelodami, Fusarium poae, Mucor hiemalis, Penicillium brevicompactum, P. digitatum, P. expansum, Rhizopus oligosporus, R. oryzae, R. stolonifer, Saccharomyces cerevisiae* and *Syncephalastrum racemosum.* About 12 fungal species (*Aspergillus niger, A. alutaceus, A. flavus, A. sulphureus, A. fumigatus, A. oryzae, A. terreus, A. tamarii, A. wentii, Penicillium expansum, P. glabrum, Fusarium poae*) with mycotoxin-producing potential were isolated from the four spice and seasoning samples at all the tested ERHs (table 3). All the four spices and seasoning samples haboured different fungal species on all media tested. Interestingly, mycoflora diversities on all spices and seasonings were predominated by *Aspergillus* followed by *Penicillium* and Mucoralean species (table 1).

The differences in diversity of fungi isolated from the spice and seasoning samples may reflect in the ability of the resident fungi in utilizing the intrinsic nutrients at the prevailing environmental conditions. Interestingly, the four spices and seasonings shared the same contaminating members of Mucorales, presumably indicative of the similar environmental conditions under which these spices and seasonings were prepared in the factory premises.

The isolation of the higher diversities of fungi at ERHs of 85% and 95% is indicative of what could happen with the changing vicissitude of the weather condition under storage. Unsurprising, eighteen ubiquitous fungal species persisted in the

| S. no | Fungi species | 75% (Initial) | 85% ERH | 95% ERH |
|---|---|---|---|---|
| 1 | *Absidia corymbifera* | ✓ | ✓ | ✓ |
| 2 | *Absidia glauca* | ✓ | ✓ | ✓ |
| 3 | *Acremonium strictum* | ✗ | ✓ | ✗ |
| 4 | *Aspergillus alutaceus* | ✓ | ✓ | ✗ |
| 5 | *Aspergillus flavus* | ✓ | ✓ | ✓ |
| 6 | *Aspergillus fumigatus* | ✓ | ✓ | ✓ |
| 7 | *Aspergillus niger* | ✓ | ✓ | ✓ |
| 8 | *Aspergillus oryzae* | ✓ | ✓ | ✓ |
| 9 | *Aspergillus striatus* | ✗ | ✓ | ✓ |
| 10 | *Aspergillus sulphureus* | ✗ | ✓ | ✓ |
| 11 | *Aspergillus tamarii* | ✓ | ✓ | ✓ |
| 12 | *Aspergillus terreus* | ✗ | ✓ | ✓ |
| 13 | *Aspergillus wentii* | ✓ | ✗ | ✗ |
| 14 | *Botrytis cinerea* | ✓ | ✗ | ✗ |
| 15 | *Cladosporium herbarum* | ✓ | ✗ | ✓ |
| 16 | *Eurotium amstelodami* | ✓ | ✓ | ✓ |
| 17 | *Fusarium chlamydosporum* | ✗ | ✗ | ✓ |
| 18 | *Fusarium poae* | ✓ | ✓ | ✓ |
| 19 | *Mucor hiemalis* | ✓ | ✓ | ✓ |
| 20 | *Nigrospora oryzae* | ✓ | ✗ | ✗ |
| 21 | *Paecilomyces variotii* | ✓ | ✗ | ✗ |
| 22 | *Penicillium aurantiogriseum* | ✗ | ✓ | ✓ |
| 23 | *Penicillium brevicompactum* | ✓ | ✓ | ✓ |
| 24 | *Penicillium charlesii* | ✗ | ✓ | ✓ |
| 25 | *Penicillium digitatum* | ✓ | ✓ | ✓ |
| 26 | *Penicillium expansum* | ✓ | ✓ | ✓ |
| 27 | *Penicillium glabrum* | ✗ | ✓ | ✓ |
| 28 | *Penicillium italicum* | ✗ | ✓ | ✓ |
| 29 | *Penicillium verrucosum* | ✗ | ✓ | ✗ |
| 30 | *Rhizopus oligosporus* | ✓ | ✓ | ✓ |
| 31 | *Rhizopus oryzae* | ✓ | ✓ | ✓ |
| 32 | *Rhizopus stolonifer* | ✓ | ✓ | ✓ |
| 33 | *Saccharomyces cerevisiae* | ✓ | ✓ | ✓ |
| 34 | *Syncephalastrum racemosum* | ✓ | ✓ | ✓ |
| | Total | 24 | 28 | 27 |

Table 2: Total list of the mycoflora resident in the four spices and seasonings after storage at 75% (Initial**)**, 85% and 95% ERHs for 7 days at 30 ± 2°C.

samples at 75-95% ERH. Namely, *Absidia corymbifera*, *A. glauca*, *Rhizopus oligosporus*, *R. oryzae*, *R. stolonifera*, *Syncephalastrum racemosum*, *Mucor hiemalis* (Mucorales), *Aspergillus flavus*, *A. fumigatus, A. niger, A. oryzae, A. tamarii, Eurotium amstelodami, Fusarium poae, Penicillium brevicompactum, P. digitatum, P. expansum*, and *Saccharomyces cerevisiae* (yeast) (table 2).In Ghana, the resident mycoflora of spices such as toxigenic *Aspergillus* and *Penicillium* species were recorded by Odamtten *et al.* (2018). Spices and herbs may be contaminated because of the conditions under which they are cultivated, harvested, microorganisms present in the processing plant, post- harvest contamination from dust and use of contaminated water and from human contact (Wirtanen and Sjoberg, 1993).

**Influence of spices and seasonings-based media on the vegetative growth of dominant *Aspergillus* species:** The various spices and seasonings (seasoned meat tenderizer, poultry seasoning, ground cinnamon and sausage spices) extracts variably supported good vegetative growth of *A. niger*, *A. flavus* and *A. fumigatus* (table 4). Seasoned meat tenderizer (290.23 ± 23.33) mg supported significantly the highest vegetative growth of *A. niger*, followed by poultry seasoning (190.42 ± 12.01) mg and ground cinnamon yielding (170.37 ± 9.00) mg, the least growth was obtained on sausage spices (130.44 ± 6.00) mg. The pH of the sausage spices media drifted to the basic during the 7 days of growth in cultures. The drift in pH is in response to the growth of the fungus, in the other media, there were slight shifted from the initial (table 4). In the case of *A. fumigatus*, vegetative growth was significantly higher in seasoned meat tenderizer (250.43 ± 8.82) mg and was closely followed by poultry seasoning (230.05 ± 3.33) mg, sausage spices (150.36 ± 5.77) mg, with ground cinnamon (80.17 ± 20.82) mg yielding the least vegetative growth (table 4). The drift in pH was in response to the growth of the fungus. Thus, was evident in ground cinnamon (pH 5.20-8.34) and sausage spices (pH 5.12-8.28) (table 4). Although the vegetative growth of *A. flavus* was not significantly different (P>0.05) on all the four spices and seasonings-based media, the best growth of *A. flavus* was obtained on poultry seasoning (80.24 ± 13.33) mg, followed by ground cinnamon, seasoned meat tenderizer and sausage spices, recording 60.47 ± 3.33 mg, 60.38 ± 26.67 mg and 50.36 ± 6.67 mg respectively in decreasing order. The pH of most media drifted to the acid side in some instances in the 7 days of growth in culture (table 4).

**Influence of spices and seasonings-based agar media on the colony diameter:** At 7 days, colony diameter growth of all test *Aspergillus* species (*A. niger, A. flavus, A. fumigatus*) differed among the spices and seasonings-based media (P< 0.001). The best colony growth of *A. niger* was obtained on seasoned meat tenderizer (85.4±5.4) mm (figure 3). This was followed by poultry seasoning (78.0±0.0) mm and then sausage spices (62.0±2.0) mm. The least colony growth was obtained on ground cinnamon (40.0±2.0) mm (figure 3). The growth of *A. fumigatus* and *A. flavus* on the formulated spices/seasonings-based solid media followed a similar trend. In that, the best colony growth of both fungi was obtained on sausage spices (84.8±1.6 mm for *A. fumigatus*) (figure 4) and (85.0±0.0 mm for *A. flavus*) (figure 5). This was followed by seasoned meat tenderizer (76.7±1.4 mm for *A. fumigatus* and 80.0±0.9 mm for *A. flavus*) and then poultry seasoning (*A. fumigatus*- 36.2±0.4 mm and *A. flavus* -72.8±0.8 mm). Ground cinnamon (22.5±2.1 mm for *A. fumigatus* and 52.2±1.5 mm for *A. flavus*) yielded the least colony growth. These fungal species therefore grew well in both liquid and solid agar media, indicative of their suitability to support growth of resident fungi.

**Preliminary mycotoxin analysis of Spice and Seasoning samples imported into Ghana:** Only sausage spices contained aflatoxin B1 (1.951 µg/kg) and aflatoxin B2 (0.552 µg/kg) but did not contain aflatoxin G1 and G2. The total aflatoxin content was 2.503 µg/kg (table 5). The rest of the samples; seasoned meat tenderizer, poultry seasoning and the ground cinnamon did not contain aflatoxins at all. The same sample of sausage spices also contained 26.470 µg/kg ochratoxin A. The rest were free of ochratoxin A (tables 6). A fortuitous condition is created whereby there is concurrent production of two mycotoxin in the same spice (tables 5).

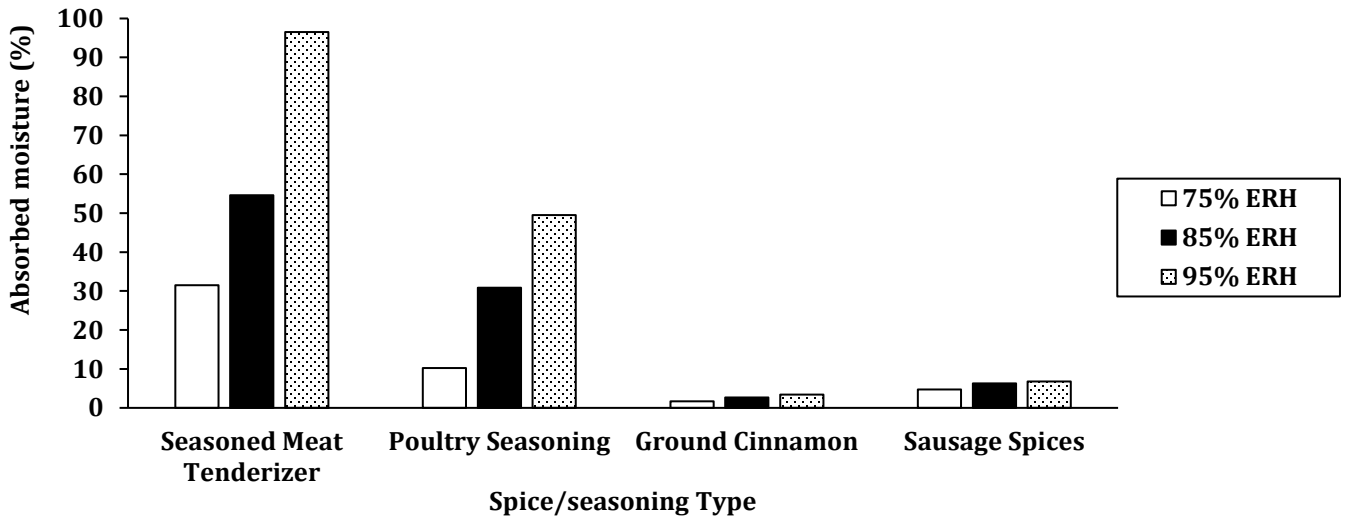


Figure 1: Moisture sorption of the selected spices and seasonings incubated at 75%,85% and 95% Environmental Relative Humidity (ERH) for 8 days at 30 °C

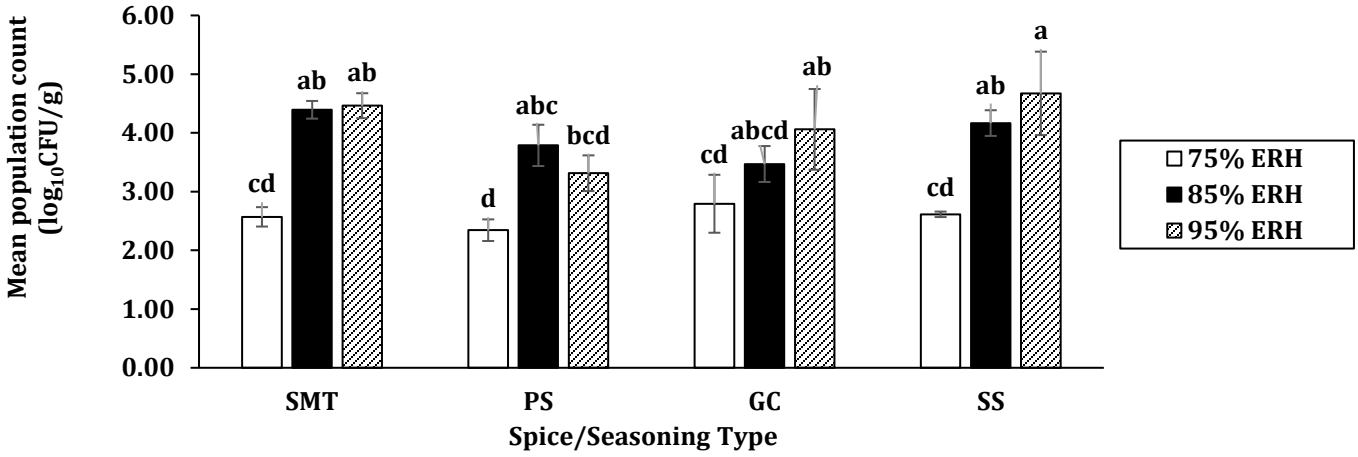


Figure 2: Mycoflora population of the indicated spices and seasonings in the initial samples and after storage at 85% and 85% ERHs for 8 days at 30 ± 2°C. Different alphabets indicate significant differences (P< 0.05) according to Duncan's Multiple Range Test. SMT: Seasoned meat tenderizer; PS: Poultry seasoning; GC: Ground Cinnamon; SS: Sausage Spice

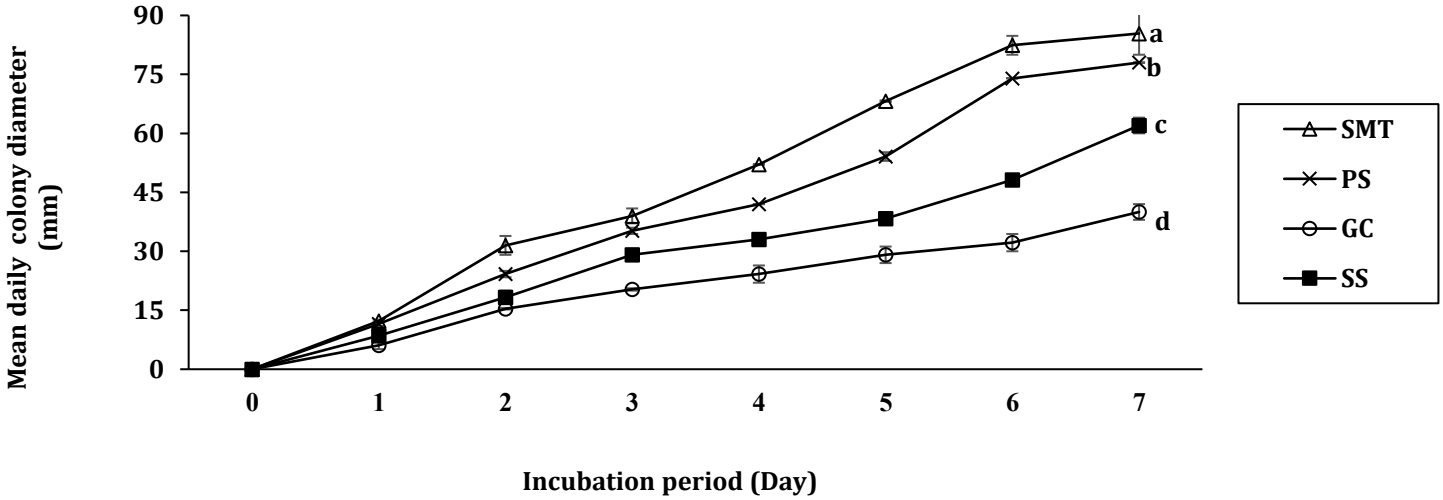


Figure 3: Colony growth of *Aspergillus niger* at 30 ± 2 °C for 7 days in the indicated agar media of the test spices and seasonings (note the variation in growth on the different media). Different alphabets indicate significant differences (P< 0.05) compared among the various media on the 7th day according to Duncan's Multiple Range Test. SMT: Seasoned meat tenderizer; PS: Poultry seasoning; GC: Ground cinnamon; SS: Sausage spices

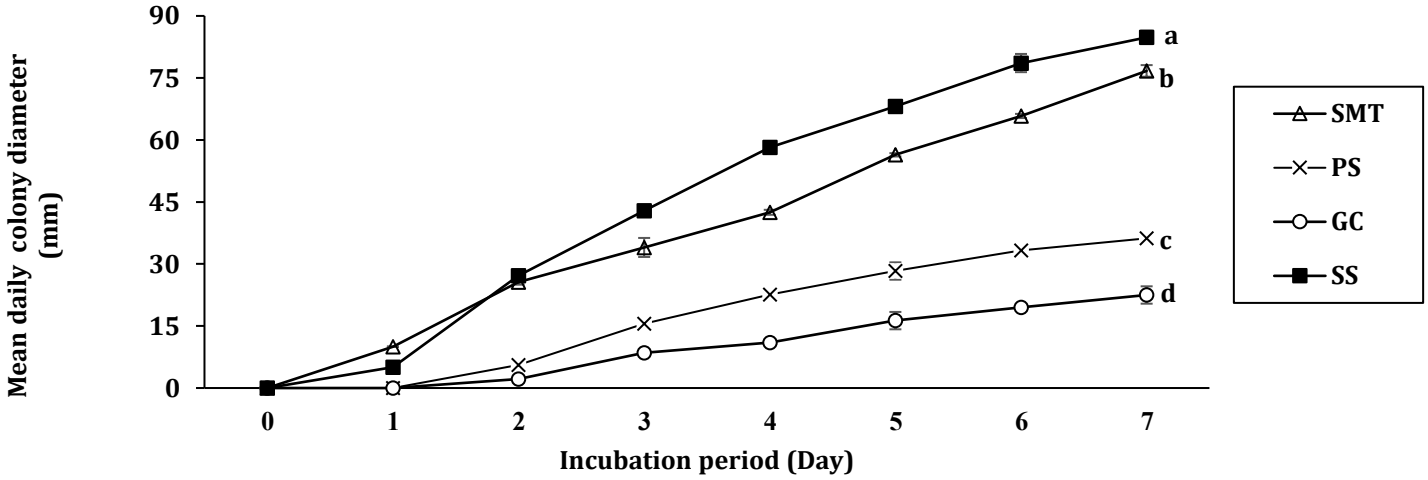


Figure 4: Colony growth of *Aspergillus fumigatus* at 30 ± 2 °C for 7 days in the indicated agar media of the test spices and seasonings (note the variation in growth on the different media). Different alphabets indicate significant differences (P< 0.05) compared among the various media on the 7th day according to Duncan's Multiple Range Test. SMT: Seasoned meat tenderizer; PS: Poultry seasoning; GC: Ground cinnamon; SS: Sausage spices

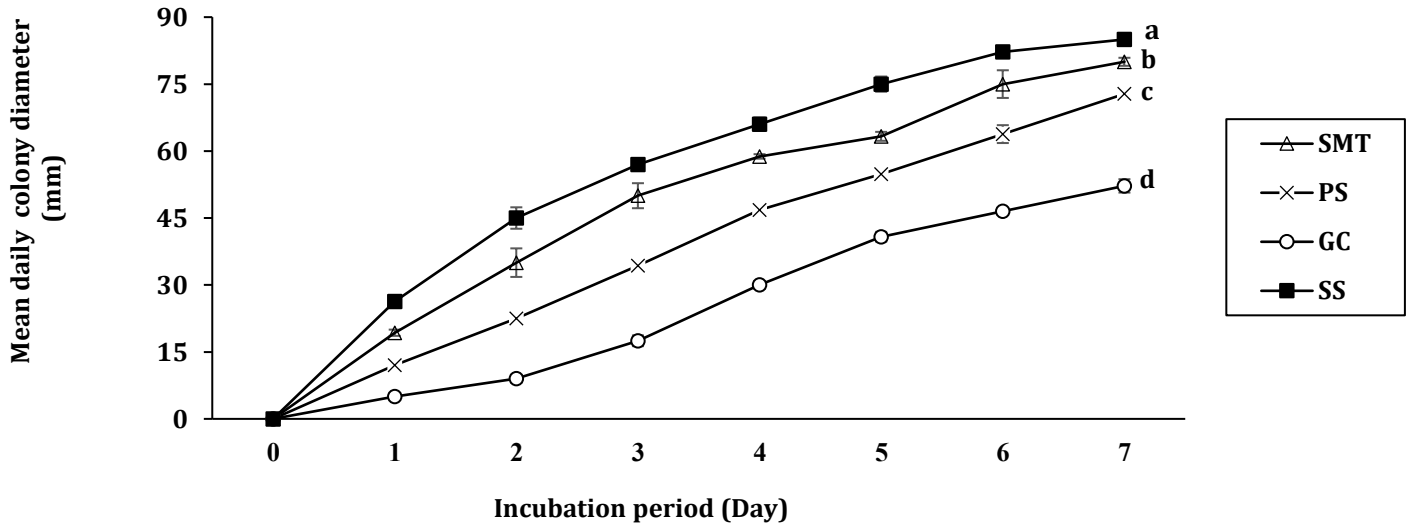


Figure 5: Colony growth of *Aspergillus flavus* at 30 ± 2 °C for 7 days in the indicated agar media of the test spices and seasonings (note the variation in growth on the different media). Different alphabets indicate significant differences (P< 0.05) compared among the various media on the 7th day according to Duncan's Multiple Range Test. SMT: Seasoned meat tenderizer; PS: Poultry seasoning; GC: Ground Cinnamon; SS: Sausage Spice.

| S. no | Fungal species | Mycotoxins |
|---|---|---|
| 1. | *Aspergillus niger* | Nigerone, oxalic acid |
| 2. | *A. alutaceus* | Ochratoxin |
| 3. | *A. flavus* | Aflatoxin |
| 4. | *A. sulphureus* | Ochratoxin A |
| 5. | *A. fumigatus* | Fumigallin |
| 6. | *A. oryzae* | Aspergillomarasmin |
| 7. | *A. terreus* | Citrinin |
| 8. | *A. tamarii* | Cyclopiazonic acid |
| 9. | *A. wentii* | Ochratoxin A |
| 10. | *Penicillium expansum* | Patulin |
| 11. | *P. glabrum* | Citromycetin |
| 12. | *Fusarium poae* | Trichothecenes A |

Table 3: List of potential Mycotoxigenic species encountered on the spices and seasonings

| Fungal species | Types of spice | PH of medium | | Dry weight of mycelium (mg) (Mean ± S.E.) |
|---|---|---|---|---|
| | | Initial | Final | |
| *Aspergillus niger* | SMT | 5.68 | 5.47 | 290.23 ± 23.33[a] |
| | PS | 5.60 | 5.56 | 190.42 ± 12.01[b] |
| | GC | 5.20 | 5.74 | 170.37 ± 9.00[b] |
| | SS | 5.12 | 8.39 | 130.44 ± 6.00[c] |
| *A. fumigatus* | SMT | 5.68 | 5.43 | 250.43 ± 8.82[a] |
| | PS | 5.60 | 5.55 | 230.05 ± 3.33[a] |
| | GC | 5.20 | 8.34 | 80.17 ± 20.82[c] |
| | SS | 5.12 | 8.28 | 150.36 ± 5.77[b] |
| *A. flavus* | SMT | 5.63 | 6.08 | 60.38 ± 26.67[a] |
| | PS | 5.55 | 3.38 | 80.24 ± 13.33[a] |
| | GC | 5.20 | 2.82 | 60.47 ± 3.33[a] |
| | SS | 5.10 | 3.56 | 50.36 ± 6.67[a] |

Table 4: Influence of Media (Spices and Seasonings) on the vegetative growth of the indicated *Aspergillus* species at 30 ± 2°C for 7 days. [1]Means (± standard error) in the same column of the same fungal species not followed by the same letter are statistically significant at 5% level, according to Duncan's Multiple Range Test. SMT: Seasoned meat tenderizer; PS: Poultry seasoning; GC: Ground Cinnamon; SS: Sausage Spice

| Sample code | Aflatoxin (µg/kg) | | | | | Ochratoxin A (µg/kg) |
|---|---|---|---|---|---|---|
| | B1 | B2 | G1 | G2 | Total | |
| **Seasoned meat tenderizer** | ND | ND | ND | ND | ND | ND |
| **Poultry seasoning** | - | - | ND | ND | ND | ND |
| **Ground Cinnamon** | ND | ND | ND | ND | ND | ND |
| **Sausage spices** | 1.951 | 0.552 | ND | ND | 2.503 | 26.470 |

Table 5: Aflatoxin and Ochratoxin A analysis of indicated Spices and Seasonings obtained from the supermarket (Marina Mall). Detection Limits: Aflatoxin B1 and B2: 0.15 µg/kg, Aflatoxin G1 and G2: 0.13 µg/kg, Ochratoxin A: 0.83 µg/kg, ND: Not Detected

It has been confirmed that, spices, seasonings, condiments and herbs are usually associated predominantly with *Aspergillus* and *Penicillium* species of which many produce mycotoxins *in vitro*. These fungal species impart mycotoxins during their growth in food and food products making food and spices unfit for human consumption. The mycotoxins with the most potential human health hazards are toxic of storage fungi in the genera *Aspergillus* and *Penicillium* and *Fusarium*. The most potent are aflatoxin B1, B2, G1 and G2 produced by *A. flavus*, *A. parasiticus* and *A. nomius*, ochratoxin A (*A. alutaceus*, *A. sulphureus*), fumonisins B1, B2, B3 and trichothecenes (produced by some *Fusarium* species), patulin (*Penicillium expansum*, *P. digitatum*) (Richard, 2008). Motloung *et al.* (2018) reported the presence of aflatoxin B1(3-19 µg/kg), aflatoxin G1 (10-11 µg/kg), Ochratoxin A (4-20 µg/kg), fumonisin B1 (104-591 µg/kg), fumonisin B2 (64-5,897 µg/kg), sterigmatocystin (11-57 µg/kg), 3-acetyldeoxynivalenol (42-46 µg/kg) and roquefortine C (17-57 µg/kg) in South African food spices. In this present study, aflatoxins (B1, B2, G1 and G2) and ochratoxin A were analyzed in the spice and seasoning samples. Only Sausage spices contained Aflatoxin B1 (1.951 µg/kg) and Aflatoxin B2 (0.552 µg/kg). Total Aflatoxin content was 2.503 µg/kg (table 5). But Aflatoxin G1 and G2 could not be detected. However, this was below the maximum permissible limit of 5.0 µg/kg for aflatoxin B1 and 10 µg/kg for total aflatoxin set by European Union for spices (EU Commission Regulation, 2010). Haruna *et al.* (2016) reported total aflatoxin in clove (>20 µg/kg) and nutmeg (11.4 µg/kg) and surprisingly, our Sausage spices used in this study contains both clove and nutmeg as part of its ingredients. On the other hand, the same samples of Sausage spices also contained 26.470 µg/kg Ochratoxin A (table 5). This Ochratoxin A value detected in Sausage Spice is above the European Union's maximum allowable limit of 15.0-20.0 µg/kg for all spices and mixtures of spices. This indicates the importance of broadening the survey of Ochratoxin A (OTA) contamination of domestic and imported spices and condiments into countries as intimated by Kumagai *et al.* (2008) and Brera *et al.* (2011).

Co-production of two potent mycotoxins in one food sample is possible and gives cause for concern because of human health implications. Co-occurrence of Aflatoxin B1, Fumonisin B, Ochratoxin A and Zearalenone in cereals dehydrated products and groundnut have been reported (Sangare-Tigori *et al.*, 2006). In Ghana, Wareing *et al.* (2001) detected sterigmat-ocystin, patulin, cyclopiazonic acid, penicillic acid, tenuazonic acid, aflatoxin in kokonte (cassava flour) while co-occurrence of high levels of aflatoxins, ochratoxins and citrinin in fermented maize dough and fumonisins were recorded (Kpodo *et al.*, 2006). The maximum permissible level of aflatoxin M1, which is the hydroxylated aflatoxin B1 (Meeting, 2001) in food is between 0.05 µg/kg and 0.5 µg/kg. Aflatoxin M1 is considered as a biomarker of aflatoxin exposure. However, aflatoxin tolerance limit differs from one food commodity and region to another. A Provisional Tolerable Weekly Intake (PTWI) of Ochratoxin A is 0.1 µg/kg (100 ng/kg) body weight, corresponding to approximately 0.014 µg/kg (14 ng/kg) body weight per day. There has been a proven correlation between hepatitis B and aflatoxins in Africa (Peers and Linsell, 1973). Liver incidence varied over a 5-fold range and was strongly associated with estimated levels of aflatoxins. There is every reason to be concern about these levels detected in the sausage spices because continuous consumption of mycotoxin is cumulative on the human tissue. The Scientific panel on Contaminants in Food Chain of the European Food Safety Authority admonishes that reduction of total dietary exposure to aflatoxins could be

achieved by reducing the number of highly contaminated foods reaching the market through more effective enforcement. The influence of the natural media prepared from the four spices on vegetative growth of the three most frequently encountered *Aspergillus* species (*A. niger*, *A. fumigatus* and *A. flavus*) tested *in vitro* indicated that all four spices/seasonings supported growth appreciably but to different extent (table 4). The results of this study indicate that the spices and seasonings used are suitable substrate for growth of mycotoxigenic fungi and further mycotoxin production as shown by Jeswal and Kumar (2015). Sausage Spice which had the least moisture sorption, supported the least of the vegetative growth of the *Aspergillus* species in the liquid medium but grew well on solid agar and contained the highest Aflatoxin B1, B2 and Ochratoxin A, but no G1 and G2 were formed (table 4-6). It is interesting to note that analysis of aflatoxin B1, B2, G1 and G2 in Seasoned meat tenderizer, Poultry seasoning and Ground cinnamon did not also yield any aflatoxins as well as Ochratoxin A, even though averagely, moisture sorption, mycelium dry weight and colony diameter were relatively high than what obtained in Sausage Spice. Sahar *et al.* (2015) found a positive correlation between moisture content in red chilies and aflatoxin content. The non-detection of mycotoxins in the Seasoned meat tenderizer, Poultry seasoning and Ground cinnamon samples could presumably be attributed to the fact that the raw spices and seasonings were stable and their formulation offered intrinsic self-protection against aflatoxin formation by *A. flavus* and Ochratoxin A by *A. alutaceus*. Ahene *et al.* (2011) and Odamtten *et al.* (2018) also attributed the non-production of aflatoxin and Ochratoxin in certain spices (Benny Beef powder, Benny crayfish powder, Remie chicken seasoning powder, Nepa Valley Jolley rice seasoning) to the self-protection offered by unspecified amount of monosodium glutamate (MSG), in addition to unspecified ionized salt and flavour enhancers and garlic.

**CONCLUSION:** This paper underscores the urgent continuous use of standard regulatory specifications to monitor quality of imported as well as locally manufactured dehydrated foods and spices to preclude the local populace from eating products laden with mycoflora and mycotoxins detrimental to their health.

**CONFLICT OF INTEREST:** The Authors declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.


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