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Research Article

Aflatoxin Detection and Identification of Moulds in Jamaican Herbal Teas

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Abstract

Tea is a highly consumed beverage in Jamaica. Jamaicans consumes a wide variety of herbal infusions; referred to locally simply as tea. Twenty-two herbal tea samples were analyzed for Aflatoxin using Immuno affinity Column Chromatography technique, and Compendium of Methods for the Microbiological Examination of Foods – Mould Counting technique for mould quantification. Mould isolates were identified using Mycological techniques. All herbal tea samples had no detectable aflatoxin (<2ppb). Mould counts ranged between <1- 2650 CFU/g. Seven types of moulds were identified including an isolate of *Aspergillus flavus* in 13.8% of the samples, and *Aspergillus montevidensis* in 18.2%. Other mould species identified were: *Acremonium spp., Paecilomyces variotii, Penicillium spp., Rhizopus microspores* and *Trichoderma spp.* respectively to be in (22.7%, 18.2%, 18.2%, 22.7% and 13.6%) of the total sample. The microbiological and mycotoxin analysis results shows that the tea samples all have satisfactory results, compliant with several recommended international specifications for herbal teas.

Keywords

Aflatoxin; Tea; Herbal Infusion;Mould; Jamaica

Introduction

The consumption of herbal tea is deeply entrenched in Jamaican culture for many generations. Traditionally, tea is any hot beverage made by steeping leaves of the Camellia sinensis plant. However, in Jamaica the term 'tea' is used to refer to both herbal infusions and the traditional tea. Jamaicans make their teas from different parts of herbal plants such as leaf, fruit, root, or bark.

The variety of herbal teas consumed in Jamaica is abundant, and includes peppermint (*Mentha piperita*), ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), soursop leaf (*Annona muricata*), cerasse (*Momordica charantia*), lime leaf (*Citrus aurantiifolia*) and lemongrass (*Cymbopogon citratus*). These herbal teas form a major part of the first meal consumed daily by many Jamaicans.

Current studies on the teas consumed in Jamaica have mainly focused on the positive medical aspect of the tea and the presence of heavy metal. However, no Jamaican study has been found that assess potential hazard in teas like aflatoxin which has been found to be prevalent in nations like: Iran, Turkey, China, and India[8].

Aflatoxin is a mycotoxin produced by two moulds: *Aspergillus flavus* and *Aspergillus parasiticus*[1]. There are several types of aflatoxins, namely: B1, B2, G1 and G2, and they differ due to variations within their chemical structures. The most common, potent, and widely studied type is Aflatoxin B1[1]. Mycotoxins are compounds produced as secondary metabolites by these moulds, and they are very heat resistant; aflatoxin has been detected in samples subjected to temperatures as high as 150 °C[10].

Aflatoxin has been linked to acute toxic responses in animals and humans (mycotoxicosis) following ingestion of contaminated food commodities[11]. In addition to mycotoxicosis, Aflatoxin is classified as a group 1 carcinogen by the International Agency for Research on Cancer [1]. Due to the toxicity of aflatoxin, it is reported at maximum limits of ppb units. The presence of aflatoxin is uniquely unavoidable in tropical countries such as Jamaica, due to the suitability of the climate which encourages mould growth and the production of secondary metabolites[11].

Moulds are ubiquitous eukaryotic organisms that are often found in food products [11]. Moulds such as: *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Absidia*, *Alternaria* and *Fusarium* are commonly found in[12]. They are naturally present in the fields where these plants grow and are a part of the normal *microflora*. Commercial food products like herbal teas are processed before being sold to consumers which results in the reduction, but not total elimination of the organisms in the final product. Moulds can grow in a wide range of environmental conditions such as low or high moisture conditions, and highly acidic conditions[12].

In Jamaica, herbal tea is consumed in both the processed and unprocessed forms. A lot of studies have revealed the presence of mycotoxins and moulds in agricultural commodities, but little is known about their presence in herbal teas consumed in Jamaica. Hence this research was done to investigate the prevalence of aflatoxin, as well as to attempt to identify moulds isolated from local tea samples.

Materials and Methods

Sampling

A total of twenty-two (22) individual samples were collected from several herbal tea manufacturers in Jamaica. The sample consisted of different types of herbal teas, which were grouped into one of five categories depending on the part of the plant they were from (fruit, flower, bark, root, and leaf) and then weighed for testing (**Table 1**).

Mould Count

Method: Compendium of Methods for the Microbiological Examination of Foods, Fifth Edition - APHA, 2015 (Chapter 21):

Apparatus/Instruments: Petri dish, glass hockey sticks/spreaders, sterile bags, analytical balance, forceps, pipettes, pipette filler, incubator.

Reagents: Dichloran Rose-Bengal Chloramphenicol Agar (DRBCA), peptone, distilled water.

1. 25 g of sample was added to 225 mL 0.1% peptone water to achieve 10-1 dilution, then homogenize.
2. 0.5 mL of the diluted sample was then aseptically pipeted unto duplicate pre-poured, solidified DRBCA plates and spread inoculum with a sterile, bent glass rod.
3. Inoculated plates were then incubated for 5 days at 25 °C.
4. Plates containing 10-150 colonies were then counted. Results were then reported as colony forming units (CFU)/g based on average counts. Plates with no colonies (i.e., 0) were reported as <1.

Mould Identification

Method: Bacteriological Analytical Manual, FDA - 2001 (Chapter 18):

Apparatus/Instrument: sterile loops, compound microscope, microscopic glass slides, cover slip, needle mounter.

Reagents: Sabouraud Dextrose (SD) Agar, Dichloran Rose-Bengal Chloramphenicol Agar (DRBCA), Lactophenol Cotton Blue, 70% lab grade ethanol.

1. Mould count plates were carefully observed, and morphology of the moulds seen growing was noted.
2. Eight mould isolates which were observed growing from more than one sample were then purified on Sabouraud Dextrose Agar or DRBC Agar.
3. Fungal specimen was added to a drop of 70% alcohol on a clean microscopic glass slide, using a sterile inoculation loop.
4. Fungal sample was then teased using a needle mounter for proper mixing of sample with alcohol.

5. The mounted slides were then stained using lactophenol cotton blue. These slides were then covered using clean sterile coverslips and then observed under microscope at 40X to note the morphological characteristics for identification.
6. Further biochemical confirmation where necessary of mould isolates was carried out using BioMérieuxVitek 2.0 automated system.

Aflatoxin Detection

This analysis was conducted using the Pesticide Residue and Mycotoxin Laboratory Unit methodology for the Determination and Semi-quantification of Aflatoxin (AF01). This method references the following:

Official Methods of Analysis of AOAC International, 19th Edition - 2012 Section No, 971.22

Apparatus/Instrument: High speed blender, ultraviolet light source, mini column lined with florisil, mini column support rack and rubber bulb.

Reagents: Benzene (reagent grade), methanol-water mix, salt solution, chloroform, acetone.

1. 50 g of sample was blended for 2 minutes with 100ml of methanol-water (80:20 w/v) solution. Then centrifuged and supernatant collected in a separating funnel.
2. 25 mL of a salt solution (600 g sodium chloride, 600 g zinc acetate, 15 mL glacial acetate in 4 L water) was added to separating funnel and vigorously shaken with supernatant. 4 mL benzene was then added and shaken for a further 2 minutes. After words the funnel was put aside to allow layers to separate. The bottom layer was discarded.
3. 0.25 mL of the top layer was then transferred to the top of a mini column (containing florisil and packing material) attached to a vacuum. Column was then washed with 5 mL of hexane-acetone (80:20 v/v) solution. This was repeated four times.
4. After the wash solution has evaporated from the column, it was placed under U.V for observation. The presence of blue-fluorescent band in the column indicated the presence of aflatoxin.
5. Steps 3 to 4 was repeated using 0.5 mL and 1 mL portions of the top layer in separating funnel.
6. Band appearance in the 0.25 mL portion was equivalent to >16 ppb, 0.5 mL is 8-16 ppb and in 1 mL it is 3.2-8 ppb. No band appearance is reported as <2 ppb.

Results and Discussion

Twenty-two (22) herbal tea samples were analysed, consisting of 72.7% leaf samples, 13.6% fruit, and 4.5% root, flower, and bark derived samples. All samples were found to have no reportable Aflatoxin (<2ppb).

All five categories of herbal tea samples had average mould counts ranging from as low as 30-1770 CFU/g (**Figure 1**). This is below the maximum allowable from the European Union and Turkey guidelines. The European Union and Turkey stipulates a count of 1×10^6 CFU/g or 1×10^5 CFU/g respectively 14&15. These low counts are possible indication of successful local industry practices during processing, transport, and storage, keeping parameters like moisture in packaged products low, to deter mould proliferation.

However, the mould count deviation for fruit and leaf samples were wide (**Figure 1**). This may be due to different moisture levels in the individual samples, as well as the initial microbial load of raw material used by respective manufacturers.

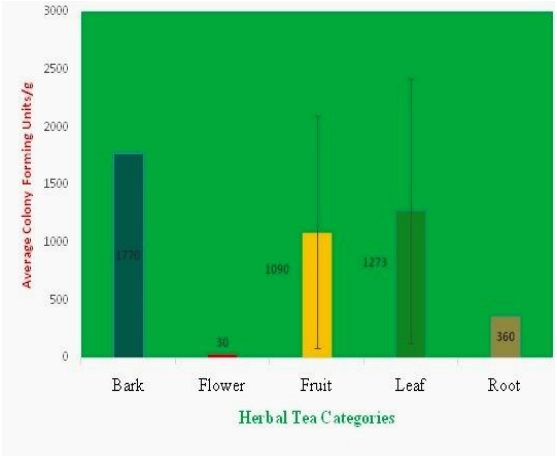

Figure 1: Average Mould Count (CFU/g) of each Category of Herbal Teas

Some herbal species have innate properties which are capable of deterring mould growth as observed. The reduced water activity within these batch of teas could have allowed for increased activity of the natural compounds found in these teas. *Psidium guajava* (guava) has displayed strong antimicrobial activity in studies done on terpinene and pinene aqueous extracts of the plant's leaves [9].

Another study carried out on *Pimenta dioica*in which preliminary phytochemical analysis on extracts showed triterpenes or steroids compound

to be the predominant active compounds. These extracts displayed antifungal activity against a range of medically significant moulds[2]. Similarly, studies on the extracts of *Azadirachta indica* (neem), demonstrated characteristic antifungal activities[13].

The overall mould counts of all twenty-two samples assessed would be readily accepted within high tea consuming markets like the EU and Turkey. This is due to the overall low mould counts being observed. This may be an indication of not just antifungal properties of some of these tea plants, but also good production processing within our local manufacturing plants.

Table 1: Aflatoxin Levels and Mould Counts determined in Herbal Tea Samples and their Categories and Codes.

Category	Herbal Sample	Codes	Aflatoxin (ppb)	Mould Count (CFU/g)
Fruit	Bissy	HT-1	<2	1800
Leaf	Cerasee	HT-2	<2	510
Leaf	Guava Leaf	HT-3	<2	< 1
Fruit	Pimento	HT-4	<2	<1
Leaf	Guinea Hen	HT-5	<2	2590
Leaf	Neem	HT-6	<2	<1
Leaf	Rosemary	HT-7	<2	10
Leaf	Moringa	HT-8	<2	2400
Flower	Chamomile	HT-9	<2	30
Leaf	Soursop+Honey	HT-10	<2	250
Fruit	Apricot Passion	HT-11	<2	380
Leaf	Moringa	HT-12	<2	280
Leaf	Cerasee	HT-13	<2	2590
Leaf	Lemon Grass	HT-14	<2	2420
Root	Ginger	HT-15	<2	360
Leaf	Cerasee	HT-16	<2	480
Leaf	Lemon Grass	HT-17	<2	290
Bark	Cinnamon	HT-18	<2	1770
Leaf	Soursop	HT-19	<2	240
Leaf	Soursop+Moringa	HT-20	<2	2650
Leaf	Moringa	HT-21	<2	540
Leaf	Peppermint	HT-22	<2	2570

Table 2: Mould isolates identified from Herbal Tea samples.

Mould Isolates	Herbal Tea Sample Codes	Mould ID
A	7,8,4,22,20	*Acremonium spp.*
B	2,5,8,9,13,14,15	*Rhizopus microsporus*
C	5,1,2,15	*Paecilomycesvariotii*
D	1,2,15,8	*Penicillium spp.*
E	1,2,15,11	*Aspergillus montevidensis*
F	2,3,17	*Trichoderma spp.*
G	13,15,6	*Aspergillus flavus*
H	2,15,19,10,12	*Rhizopus microspores*

Although no aflatoxin was detected in any of the twenty-two samples of this project, the mould genus *Aspergillus* which is a known producer of the mycotoxin was identified amongst the eight moulds isolated.

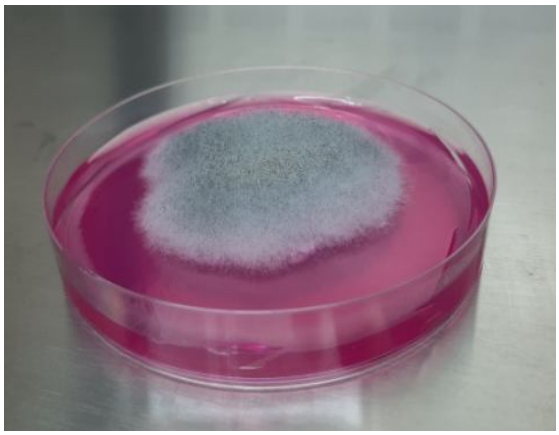

Figure 2: *Mould Isolate B - Rhizopus microsporus on DRBC agar.*

Figure 3: *Mould Isolate E - Aspergillus montevidensis on SD agar.*

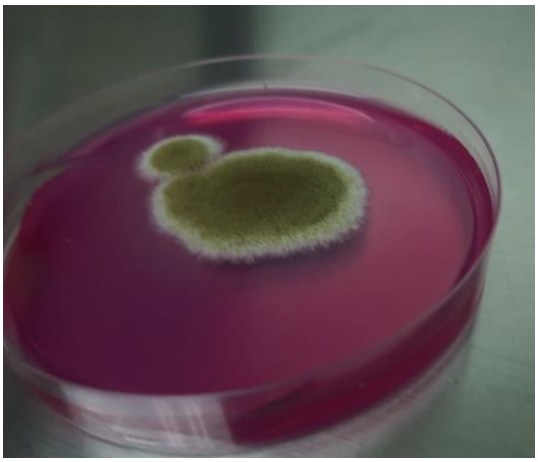

Figure 4: *Mould Isolate G - Aspergillus flavus on DRBC agar.*

A total of eight (8) isolates were identified from across the twenty-two sample, four of them were. The presence of this mould specie gives a clear indication that the possibility of aflatoxin occurring in the tea sample is likely. However, if aflatoxin was being produced in the samples from which it was isolated, the concentration is negligible. Hence, it would not have been detected when checks were being made for the aflatoxin.

The other identified *Aspergillus* mould specie was *A. montevidensis.* This specie is not known to produce aflatoxin or any other secondary metabolite which is harmful to humans [4].

Studies have shown that *Penicillium puberulum* isolated, has been found to be an aflatoxin producing specie of this mould genus[5]. Additionally, a study of metabolites produced by several strains *Rhizopus microsporus* reveals that it is a producer of the mycotoxin rhizoxins through bacterial endosymbiosis[7]. Similarly, rhizoxins are highly toxic hepatotoxin like aflatoxins.

Some species of Acremonium isolated can produce mycotoxins that are cytotoxic, nephrotoxic and have tremorgenic effects if ingested in significant amounts. However, cases of Acremonium mycotoxicosis are limited to livestock and other animals as no human cases have been reported. *Paecilomyces variotii* belongs to a family of moulds not known to produce any mycotoxin of significance, but occasionally cause clinical disease like keratitis which is the inflammation of the cornea. Similarly, *Trichoderma spp.* of moulds is rarely pathogenic but can cause clinical disease like endocarditis and pulmonary infection in immune compromised individuals[6].

Conclusions

It was determined that no aflatoxin was present in any of the twenty-two samples analyzed from local tea manufacturers. However, the mould specie *Aspergillus flavus* which produces aflatoxin was found to be present in 12.5% of the total samples analyzed. A total of eight moulds isolates were identified, which consisted of seven (7) different genera and four (4) identified species. The average mould counts across the five categories of herbal teas analyzed ranged between 30-1770 CFU/g. These counts are well within the existing recommended maximum mould counts specified by the European Union and Turkey, which are two high tea consuming countries. There is need for further studies to be conducted considering a larger sample size, increased mould isolate identification and assessment for other mycotoxins that may be present in these highly consumed products. It is also noteworthy that this research can form the framework around establishing a local standard with specifications for herbal teas within the local market.

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Conflict of interest

The authors declare that there are no conflicts of interest to the present work.

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