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Risk Assessment of Mycotoxins and Predictive Mycology in Sri Lankan Spices: Chilli and Pepper

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Abstract

This research contributes to a better understanding of the mycotoxin problem associated with two important spices in world trade;, chilli and pepper by a multidisciplinary approach including analytical chemistry, risk assessment, food chemistry and predictive mycology. More specifically, this work provides important insights in mycotoxin contamination of these spices and associated risks in Sri Lanka. Firstly, a simple extraction method based on the QuEChERS approach was developed and successfully validated for the simultaneous determination of multiple mycotoxins using an advanced chromatographic technique, LC-MS/MS. The method was applied on complex spices for quantitative screening of seventeen mycotoxins. In addition to the classical aflatoxins and ochratoxin A, the spices were also found to be contaminated with several other toxicologically significant mycotoxins. Chilli samples (87%) were more frequently contaminated with mycotoxins than peppers (65%). Subsequently, the mycotoxins screening results and the collected consumption data were integrated in a quantitative risk assessment study. The results showed that AFB1 exposure via chilli consumption is of a public health concern in Sri Lanka, pepper is of lesser extent a risk due to the lower consumption. The toxigenic mould characterization in black peppers showed that Aspergillus flavus and/or Aspergillus parasiticus were the predominant moulds (73%) found, with considerable contamination (60%) of Penicillium spp. and A. niger. Furthermore, predictive mould growth models on peppercorns were developed at three temperatures and seven water activity levels for both A. flavus and A. parasiticus isolates. Based on the research, suitable storage conditions for black peppercorns were suggested and the way forward in managing the risk towards mycotoxins posed by the consumption of these two spices in Sri Lanka.

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1. Introduction

Sri Lanka, "The Pearl of Indian Ocean" in South Asia is also known as "Spice Island", where highly priced spices are produced, consumed and exported. Spices are well known for imparting flavour, colour, aroma in diverse cuisines and for therapeutic properties. However, their quality is often compromised. As a developing country Sri Lanka has its own limitations in producing high quality spices for local consumption and in compling with trade regulations enforced by the importing countries. Moreover, as a tropical nation the prevailing climatic conditions, while supporting the spice crop development could also be highly favorable for mould infestation and mycotoxin contamination in the field or during post-harvest practices. Mycotoxins are toxic secondary metabolites produced by diverse[1]. filamentous fungi. Like many other foods, spices could also be contaminated with moulds and mycotoxins affecting their safety and quality. Hence, this research work is carried out in order to identify the actual situation in moulds and mycotoxin contamination in spices and to perform a quantitative risk assessment of mycotoxins in Sri Lanka, given the limited information on the mycotoxin issues therein. In this study, chilli (Capsicum annum L.) and pepper (Piper nigrum L.) were selected since these two are important spices of world trade and consumption.

2. Methodology

First, a reliable and rapid method was developed based on a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction procedure for the determination of multiple high-performance liquid chromatography mycotoxins in spices, chilli, black and white peppers.[2] Tandem mass spectrometry (HPLC-MS/MS) was used for the quantification and confirmation of chemically diverse mycotoxins. Mycotoxins were extracted from the hydrated spices using acidified acetonitrile, followed by partitioning with NaCl and anhydrous $MgSO_4$ excluding the use of dispersive-solid phase extraction. Electrospray ionization at positive mode was applied to simultaneously detect all the mycotoxins in a single run time of 20 min. Multiple reaction monitoring mode, choosing at least two abundant fragment ions per analyte was applied. Recoveries (75 to 117%) were in accordance with the performance criteria required by the European Commission (EC, 401/2006). The limit of quantification (LOQ) ranged from 2.3 to 146 μg/kg. The method LOQ meets the maximum levels (MLs) of the two regulated mycotoxins, aflatoxins and ochratoxin A (OTA) in spices hence, it could be used for the purpose of enforcement of the EU MLs. The validated method was finally applied to screen mycotoxins in chilli and pepper samples collected from Sri Lanka. Chilli is mainly imported from India, while the pepper is cultivated in Sri Lanka itself.

After the screening and measuring the status of the contamination of available spices, statistical analysis was performed to identify significant differences between region of sampling, form of spices (e.g. flakes, whole, powder, etc). Also a quantitative risk assessment (deterministic and probabilistic) of mycotoxins due to the consumption of chilli and black pepper in Sri Lanka was conducted. A food frequency questionnaire was administered in order to collect the data on consumption of spices by households in the Northern and Southern region (n=249).

In a next step, the growth and mycotoxin production of an A. parasiticus and three A flavus isolates in whole black peppercorns using a full factorial design with seven water activity (aw) (0.826-0.984) levels and three temperatures (22, 30 and 37°C) were used to develop predictive models. Growth rates and lag phases were estimated using linear regression. Several secondary kinetic models were assessed for their ability to describe the radial growth rate as a function of individual and combined effects of aw and temperature.*Tables*

All tables should be numbered with Arabic numerals. Every table should have a caption. Headings should be placed above tables, left justified. Only horizontal lines should be used within a table, to distinguish the column headings from the body of the table, and immediately above and below the table. Tables must be embedded into the text and not supplied separately. Below is an example which the authors may find useful.

3. Results, Discussion, Conclusions and Recommendations

The screening of the spice samples reveals the characterization of toxigenic moulds and occurrence of multiple mycotoxins in Sri Lankan peppers [3]. Characterization of the moulds was carried out in *Aspergillus flavus* and *parasiticus* agar (AFPA) and malt extract agar (MEA) in black pepper and white pepper samples. *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus niger* and *Penicillium* spp. were found to be the most dominant fungi. In total, 73% of the black pepper and 64% of the white pepper samples were contaminated with *Aspergillus flavus* and/or *Aspergillus parasiticus* (AfAp). The frequency of occurrence of *A. niger* in black pepper was 62% with counts up to 1.3*10 CFU/g. *Penicillium* spp. were found in 61% and 55% of the black and white pepper samples, respectively. Other *Aspergillus* spp., found in peppers included, *Aspergillus terrus*, *Aspergillus tamarii*, *Aspergillus candidus*, *Aspergillus penicilloides*, *Aspergillus sydowii* and *Aspergillus fumigatus*. Mould counts in black pepper (10^{2} - 10^{4} CFU/g) were significantly higher than that of white pepper ($<10^{2}$ CFU/g) (confidence level p = 0.05). Mould contamination was compared between production steps, forms, sampling regions and grades of pepper.

Apart from the occurrence of "classical mycotoxins" of spices, aflatoxins LOQ- µg/kg) and OTA (<LOQ-79 µg/kg), other toxins including fumonisin B1 (FB1; <LOQ-135 µg/kg), sterigmatocystin (STERIG; <LOQ-49 µg/kg) and citrinin (CIT; <LOQ-112 µg/kg) were detected in black peppers (n=82). STERIG was detected in 44% of samples. In total, 63% of the aflatoxin B1 (AFB1) positives exceeded the EU ML of 5 µg/kg. Moreover, 63% of the black pepper samples were contaminated with at least one mycotoxin and 12% had more than two toxins. Mycotoxin contamination in white pepper (n=11) was significantly less compared to black pepper (confidence level p = 0.05). For comparison reasons, some pepper samples from Belgium (n=27) were also analyzed.

Further screening of the co-occurrence of multiple mycotoxins in dry chilli samples (n=86) collected from Sri Lankan markets, was performed. In addition to aflatoxins (<LOQ- µg/kg) and OTA (<LOQ-282 µg/kg), the chilli samples were [4] also found to be contaminated with STERIG (<LOQ-32 µg/kg), fumonisin B2 (FB2; <LOQ-87 µg/kg), CIT (<LOQ-2.1 mg/kg) and alternariol methyl ether (70 and 222 µg/kg). AFB1 was the predominant mycotoxin contaminating almost 77% of the samples. Remarkably, 67% of the samples exceeded EU ML of 5 µg/kg for AFB1 and 44% exceeded the EU ML of 10 µg/kg for total aflatoxins. While OTA was found in 41% of the samples notably, 38% of the total samples were contaminated with STERIG. Overall, 87% of the samples, was contaminated at least with one mycotoxin. One third of the chilli samples were contaminated with more than three different mycotoxins. Co-occurrence of different mycotoxins, AFB1-OTA (36%), AFB1-STERIG (28%), OTA-AFB1-STERIG (17%) and AFB1-FB2 (14%) was found in different forms of chillies. Higher frequency of mycotoxins co-occurrence found in the processed chillies such as flakes and powder could be due to the fraudulent usage of low quality grade chilli pods for processing.

Mean exposure to AFB1 in the North (3.49 ng/kg BW/day) and South (2.13 ng/kg BW/day) have exceeded the proposed tolerable daily intake (1 ng/kg BW/day) due to chilli consumption at the lower bound deterministic scenario, while exposure to OTA was below stated toxicological values. Dietary exposure to other mycotoxins, FB1, FB2, STERIG and CIT due to spices were estimated. Margin of exposure estimations at the mean exposure to AFB1 were remarkably lower due to chilli (45-78) than for pepper (2,315-10,857). Moreover, the hepato cellular carcinoma (HCC) risk associated with the mean AFB1 exposure through chilli at the lower bound was 0.046 and 0.028 HCC cases/year/100,000 based on the North and South consumption distribution, respectively. AFB1 exposure via chilli should be considered as a high public health concern in Sri Lanka due to both high mycotoxin concentration and high consumption.

Based on the predictive modelling, optimum temperature (28-33°C) and a_w (0.93-0.99) were estimated by multi-factorial cardinal model for both in pepper (*A. parasiticus* and three *A flavus* isolates). Following the growth study, production of aflatoxins, STERIG and OMST were analyzed using LC-MS/MS. Absence or minimal production of mycotoxins in peppers following heavy mould growth suggests that pepper constituents in interfere with the mycotoxins biosynthesis at certain levels, with no or marginal influence on fungal growth and sporulation. The predictive growth models developed in this study could serve as a tool for the prevention of mould growth, which could potentially control the spice spoilage and accumulation of mycotoxins in black pepper.

4. Recommendations

Finally, recommendations are made to come to an improvement of the situation in Sri Lanka and to decrease the human health pressure of mycotoxins in spices. Two routes are formulated, one for pepper, cultivated in Sri Lanka and one for chillies, imported from mainly India (figure 1).

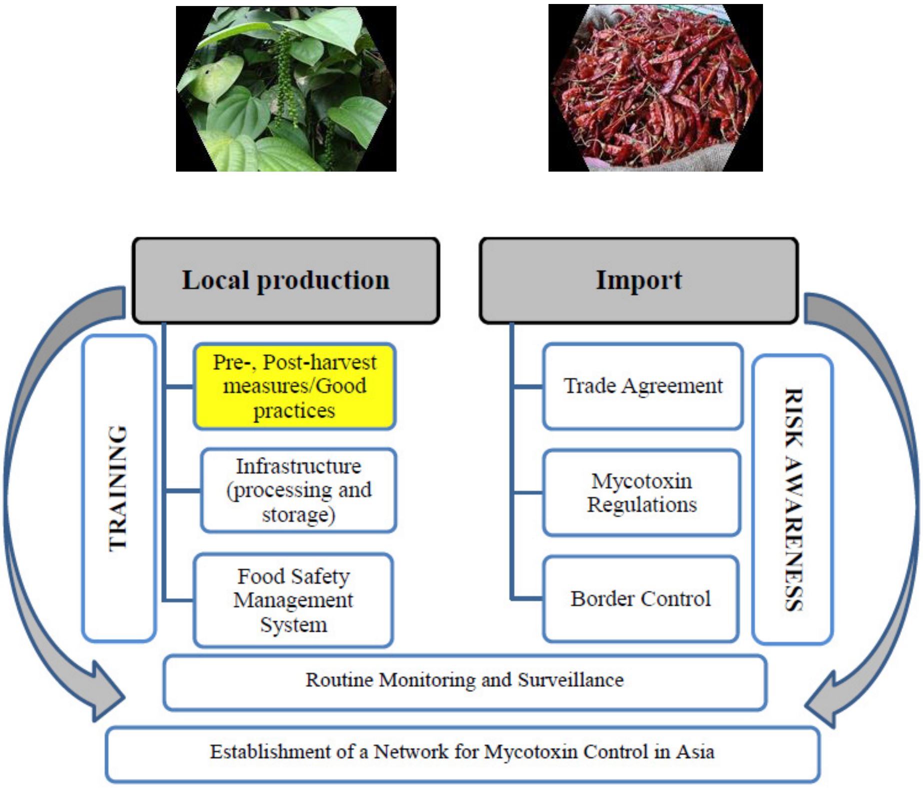

Fig. 1. Way forward improving the management the mycotoxin contamination related to chilli and pepper in Sri Lanka..

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