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Isolation, identification and molecular characterisation of aflatoxigenic Aspergillus species from dried traditional foods commonly sold at Bulawayo Market

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Abstract

The presence of aflatoxin producing *Aspergillus spp* in selected traditional foods sold on the Bulawayo open market in Zimbabwe was investigated. Ten samples of each of the following commodities were bought from the market; dried groundnuts, dried cowpeas, dried maize, dried cowpea leaves, dried mopane worms and dried *Cleome gynandra* leaves. The moisture content of these commodities was determined by weighing out 5 g of part of each sample followed by drying at 100°C until a constant weight was reached. The moisture content of the samples ranged from 7.0 to 7.6% for the mopane worms, 3.4 to 8.2% for *Cleome gynandra* 7.6 to 11.3% for dried cowpea leaves, 5.1 to 7.1% for maize, 4.2 to 4.7 for groundnuts and 4.0 to 7.1% for dried cowpeas. The other portion of the samples was then plated on petri dishes containing Sabouraud Dextrose Agar (SDA) and incubated at 25-28°C . A total of 35 isolates was obtained A polyphasic approach consisting of morphological, chemical and molecular characterisation was applied for the identification of the aflatoxigenic strains. Four distinct morphological groups were found and were classified as *Aspergillus parasiticus* (57%) green colonies, *Aspergillus niger* (17%) black colonies, *Aspergillus tamarii* (17%) brown colonies and *Aspergillus flavus* (8%) yellowish green colonies. The presence of aflatoxins was determined colometrically using Yeast Extract Sucrose agar (YES) at 28°C for 3-7 days followed by the use of ammonia gas A total of 19 isolates turned pink-red colour indicating the presence of the mycotoxin. These isolates were from mopane (10), cowpeas (5), groundnuts (3) and *Cleome gynandra* (1). The rest of the samples had no colour change on the reverse side of the plates. Some isolates that showed positive results on the ammonia vapour reaction but gave negative results on Coconut Agar Medium (CAM) where they did not produce any fluorescent ring around the colonies. Thin layer chromatography (TLC) was then used to determine the identity of the aflatoxins i.e. blue (B) or green (G). These results were validated by using DNA primers of the structural genes, *aflD* (nor-1), *aflM* (ver-1) and *aflP* (omt-1) and the regulatory gene *aflR* to discriminate between aflatoxigenic and non-aflatoxigenic strains by amplifying DNA of the fungal strains. None of the isolates produced all the four genes involved in the aflatoxin biosynthetic pathway although they had shown positive results on the biochemical tests. A total of 17 isolates showed that they were aflatoxin producers after the biochemical and molecular analysis. The aflatoxin producers were from mopane worms, dried cowpeas, groundnuts and dried *Cleome gynandra* leaves. The investigation showed that dried traditional foods were contaminated by *Aspergillus* and this condition may likely be due to mishandling, coupled by prevailing environmental conditions from the packaging to the selling points.