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LAMP-PCR detection of ochratoxigenic *Aspergillus* species collected from peanut kernel

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ABSTRACT. Over the last decade, ochratoxin A (OTA) has been widely described and is ubiquitous in several agricultural products. Ochratoxins represent the second-most important mycotoxin group after aflatoxins. A total of 34 samples were surveyed from 3 locations, including Mecca, Madina, and Riyadh, Saudi Arabia, during 2012. Fungal contamination frequency was determined for surface-sterilized peanut seeds, which were seeded onto malt extract agar media. *Aspergillus niger* (35%), *Aspergillus ochraceus* (30%), and *Aspergillus carbonarius* (25%) were the most frequently observed *Aspergillius* species, while *Aspergillus flavus* and *Aspergillus phoenicis* isolates were only infrequently recovered and in small numbers (10%). OTA production was evaluated on yeast extract sucrose medium, which revealed that 57% of the isolates were *A. niger* and 60% of *A. carbonarius* isolates were OTA producers; 100% belonged to *A. ochraceus*. Only one isolate, morphologically identified as *A. carbonarius*, and 3 *A. niger* isolates unstably produced OTA. A polymerase chain reaction (PCR)-based identification and detection assay was used to identify *A. ochraceus* isolates. Using the primer sets OCRA1/OCRA2, 400-base pair PCR fragments were produced only when genomic DNA from *A. ochraceus* isolates was used. Recently, the loop-mediated isothermal amplification assay using recombinase polymerase amplification chemistry was used for *A. carbonarius*

and *A. niger* DNA identification. As a non-gel-based technique, the amplification product was directly visualized in the reaction tube after adding calcein for naked-eye examination.

Key words: *Arachis hypogaea*; Black aspergilla; Ochratoxins; Loop-mediated isothermal amplification; Polymerase chain reaction

