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Natural occurrence of Ochratoxin A and toxigenic *Aspergillus ochraceus* strains in dry fruit slices of quinces from Jammu and Kashmir

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ABSTRACT : Ochratoxin A was detected for the first time as a natural contaminant in dry fruit slices of quinces (*Cydonia oblonga* Mill.) collected from different markets of Jammu and Kashmir state. Investigations revealed that 32% of the examined samples were positive for this toxin and the level of contamination recorded was up to l.63 mg/kg. Frequency level of toxigenic *Aspergillus ochraceus* isolates recovered from the surface of this dry fruit was low but they could produce up to 3.4 mg/l of ochratoxin A on a semisynthetic medium.

Key words : Ochratoxin A, *Aspergillus ochraceus,* dry fruit slices, quinces

Ochratoxin A (OTA), a secondary mould metabolite, produced chiefly by *Aspergillus ochraceus* Wilhelm has been reported to be nephrotoxic, hepatotoxic, immunosuppressive, teratogenic and genotoxic in animal studies (Krogh, 1978). Besides *A. ochraceus* isolates, some other notable fungal species recorded to be OTA producers are *Aspergillus ostianus, A. melleus, A. alliaceus, A. petrakii, A. sclerotiorum, A. sulphureus, A. niger* var. *niger, A. foetidus, A. awamori, A. albertensis, A. carbonarius, A. auricomus, A. wentii, Penicillium viridicatum, P. aurantiogriseum, P. verrucosum, P. cyclopium, P. commune, P. palitans, P. purpurescens, P. variabile* and *P. chrysogenum* (Krivobok *et al.,* 1995; Varga *et al.,* 1996).

Toxicity of OTA is being increasingly implicated in human and animal pathology, both in the developed and developing countries. Endemic nephropathy in the Balkans, particularly in the rural zones of Bulgaria, Croatia, Slovenia, Bosnia, Romania and Yugoslavia is believed to be on account of high exposure of ochratoxin (WHO, 1979). OTA has also been detected in the blood samples of people living in Germany, France, Scandinavia, Canada, Japan and North Africa (Creppy *et al.,* 1995). Around the globe, several investigators have demonstrated natural occurrence of OTA in diverse food and feed commodities (Krishnakumari and Nusrath, 1987; Veldman *et al.,* 1992; Wood, 1992; Oyelami *et al.,* 1996; Zimmerli *et al.,* 1996). However, meagre information is available on the natural contamination of dry fruits with OTA (Bilgrami, 1984). With this objective in mind, we decided to assess OTA contamination in dry fruit slices of quince, an important rosaceous pome fruit of J&K state, which is valued both for its religious and commercial significance. From our previous investigations, we observed that warm humid conditions prevailing during dehydration (sun-drying) and the existing faulty storage practices together render this dry fruit suitable for the growth and proliferation of various surface moulds (Sharma and Sumbali, 1996). Therefore, *Aspergillus ochraceus* isolates recovered from dry fruit surface were also screened for OTA production in mycological media.

MATERIALS AND METHODS

Isolation of *A. ochraceus*

Samples obtained from various dry fruit markets of the state were subjected to surface washing and agar plate technigues (Muskett, 1948; Christensen, 1957) for the recovery of associated *A. ochraceus* isolates. Media used were Czapek's agar and malt extract agar supplemented with streptomycin sulphate (0.06g/1) and Rose Bengal (0.20g/1).

Culture

Fresh cultures of *A. ochraceus* isolates were inoculated individually in Erlenmeyer flasks (250 ml capacity) containing 100 ml of SMKY medium (sucrose 200 g; $MgSO_4$. $7H_2O$ - 0.5 g; KNO_3 - 3.0 g; yeast extract - 7.0 g; distilled water - 1000 ml). Incubations were static at 25°C for 12 days.

Extraction and analysis

At the end of incubation period, contents of the flasks were filtered and the culture broth was extracted

twice with 25 ml portions of chloroform in separating funnels. Chloroform extracts were evaporated to dryness on a steam bath. One ml of chloroform was added to each sample, of which known amount was spotted on a thin layer chromatography (TLC) plate coated with silica gel. Standard solution of Ochratoxin A was also spotted on the same plate and developed in toluene : ethyl acetate : 90% formic acid (5:4:1 v/v). Location of OTA on TLC was accomplished with shortwave UV light (254 nm). Chemical confirmation of OTA was performed by exposing the plates to ammonia fumes for 15 minutes which changed blue green fluorescent spot to deep blue colour (Davis *et al.*, 1969). For quantitative estimation, detected spots of OTA were eluted with methanol and the concentration was determined spectrophotometrically according to molar extinction coefficient (E) which is 5,550 at 333 nm for OTA (Bacha *et al.*, 1988).

Dry fruit slices of quinces were analysed for OTA contamination by following Stoloff *et al.* (1971). Confirmation and quantitative analysis was done by the method described for liquid culture.

RESULTS AND DISCUSSION

Thirteen isolates of *A. ochraceus* recovered from surface of dry fruit slices of quinces were screened for their ability to produce OTA in SMKY medium (Table 1). Although just 23% of the isolates were found to be capable of producing OTA, yet the range of production was quite high varying from 1.6 to 3.4 mg/l. Bilgrami (1984) also recorded high level of OTA production from dry fruit isolates of *A. ochraceus* in semi-synthetic medium. Ochratoxin appears to be biosynthesized by coupling of a phenylalanine moiety, which probably arises through the shikimic acid pathway (Steyn and Holzapfel, 1967).

Twenty five market samples of dry fruit slices were also investigated for natural occurrence of OTA contamination. Results depicted in Table 2 show that 32% of the samples were OTA positive and the level of contamination varied from 0.575 mg/kg to 1.630 mg/kg of the dry fruit. High level of OTA contamination may be due to ubiquitous nature of *A. ochraceus* spores and its dominant association with this dry fruit as evidenced by previous investigation (Sumbali and Sharma, 1997). The presence of many other *Aspergillus* and *Penicillium* species recorded alongwith *A. ochraceus* (Sumbali and Sharma, 1997), is also of ecological significance.

Table 1. Production of Ochratoxin A by *Aspergillus ochraceus* isolates in culture medium

Toxigenic *A. ochraceus*	Amount of OTA produced (mg/l medium)
A 0 - 1	1.6
A 0 - 2	3.4
A 0 - 3	2.4

Number of *A. ochraceus* isolates screened - 13.

Table 2. Natural occurrence of Ochratoxin A in dry fruit slices of quinces (*Cydonia oblonga* Mill.)

Positive sample number	Amount of OTA detected (mg/kg)
1	0.725
2	1.630
3	1.280
4	0.500
5	0.575
6	0.865
7	0.830
8	1.165

Number of market samples analysed = 25.

OTA has been detected in various other agricultural commodities also (FAO, 1982), but there are no legal levels of acceptability recognised for this compound or any of its natural derivatives in spite of the toxicological significance. Although consumption of dry fruit slices of quinces is very less in comparison to cereals and other agricultural commodities in which sufficient amount of OTA have been found, yet its long range adverse effects on the health of consumers cannot be ruled out. Therefore, the present investigation impresses upon the need for routine monitoring of this dry fruit during storage and marketing, especially during warm and humid seasons when fungal infestation and concomitant production of mycotoxins is at the maximum.

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