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Natural Deoxynivalenol Contamination of Corn Produced in Golestan and Moqan Areas in Iran

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ABSTRACT

Deoxynivalenol contamination was determined in corn produced in Golestan and Ardabil (Moqan) Provinces, Iran, in 2004-2005. Samples were collected from different stages of production, including before harvest, at harvest, post harvest as well as after drying. Ground sub samples were extracted with water; each extracted sample was cleaned up through an immunoaffinity column. Deoxynivalenol was estimated through reversed-phase High Performance Liquid Chromatography (HPLC). The linearity of standard curve for 50 -10,000 ng ml^{-1} of standard solutions was proved (R^2= 0.9999). Detection limit was 10 ng g^{-1}. Recovery of the method for 1,000 and 500 ng g^{-1} spiked samples was 73.5% and 93.5% (n= 5). Deoxynivalenol contamination was found in 76.7% of samples in the range of 54.4-518.4 ng g^{-1}. The mean of contamination was 116.25 ng g^{-1}. This is the first report of natural DON contamination of corn from Iran.

Keywords: Corn, Deoxynivalenol, HPLC, Iran, Natural contamination.

INTRODUCTION

Deoxynivalenol is one of the trichothecenes produced by *Fusarium* species. *Fusarium graminearum* (Teleomorph: *Gibberella zeae*) isolates are considered as major producers of deoxynivalenol (DON), nivalenol (NIV), and their derivatives including 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), 4-acetylnivalenol (4-ANIV), in wheat, barley and corn grains (Kimura *et al*., 2003). Many studies account for the deleterious effects of DON on animal and human health (CAST, 2003). DON ingestion causes acute and chronic toxicity and affecting animal feeding, behavior, as well as immune function (Rotter *et al*., 1996; Lautraite *et al*., 1997; Schlatter, 2004; Pestka *et al*., 2004). In domestic or laboratory animals, high doses of DON caused feed refusal, decreased weight gain, vomiting, gastrointestinal and dermal irritation and immunological alterations. DON reduces growth and feed consumption (anorexia) at low concentrations in the diet and induces vomiting at higher doses (Rotter *et al*., 1996). DON is known to be clastogenic (Knasmuller *et al.*, 1997). DON is reported to bind to the ribosomal peptidyl-transferase site, inhibiting protein and DNA synthesis, consequently, the exposure resulting in decreased cell proliferation (Shifrin and Anderson, 1999). This mycotoxin is a potent protein synthesis inhibitor that can significantly alter humoral immunity, cell-mediated immunity, and host resistance in a variety of experimental animal models (Pestka and Bondy, 1990).

Golestan and Moqan areas are located in the North and Northwestern Iran with favorite conditions for *Fusarium* growth.

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Fusarium head blight of wheat and barley are two important diseases in Golestan Province (Golzar *et al.*, 1998). Different *Fusarium* species were reported as the causal agents of corn ear rot and as the mycoflora of corn seeds. *F. oxysporum, F. semitectum* and *F. subglutinans* have been isolated from maize in Iran (Gerlach and Ershad, 1970). *F. proliferatum* and *F. verticillioides* were also among the components of the mycoflora of corn seed in Iran (Boujari and Ershad, 1993; Ershad, 1995). *F.monilifrome* and *F.prolifratum* were isolated from rotted ears collected from experimental as well as from seed production fields in Sari and Karaj areas that artificially caused ear rot (Zamani and Alizadeh, 2000). A study on mycoflora of maize harvested in the main production areas of Iran in 2000 showed that *Fusarium* species were predominant (38.5%), *F. verticillioides* being the most predominant (52% of the total isolates). Following *F. verticillioides*, *F. proliferatum* was the most prevalent among the species. Other species present at low incidence levels were *F. acuminatum, F. scirpi, F.equiseti, F. semitectum, F. nygamai* and *F. culmorum* (Ghiasian *et al*., 2004).The research reported here was conducted to determine the DON contamination of corn crop in the above mentioned areas.

MATERIALS AND METHODS

Reagents and Standards

DON standard was purchased from Sigma (St. Louis, MO, USA). Stock solution was prepared by dissolving the solid standard in methanol (1 mg ml^{-1}). More diluted standard solutions for calibration (50, 100, 500, 1,000, 2,000, 3,000, 5,000 and 10,000 ng ml^{-1}) were prepared through evaporation of aliquots (under N_2 stream) followed by appropriate dilution with the mobile phase or methanol. The standard solutions were kept in tightly sealed vials at -20˚C. They were brought to room temperature before use. Acetonitrile, methanol and water were analytical or HPLC grade purchased from MERCK Company (Germany), DONPREP immunoaffinity columns were obtained from R-BIOPHARM RHONE LTD (Glasgow, Scotland). Filter papers (Whatman No.1) and glass microfibre (GF/A) were from Whatman (Maidstone, UK.).

Sample Preparation, Extraction and Clean-up

Sixty corn samples were collected from different locations of Golestan and Moqan areas in the North and Northwestern Iran, during 2004-2005. Each 5-10 kg sample was finely ground, using Romer Series ΠTM mill, (MO, USA) and well mixed. A 100 g subsample was taken from each main (ground) sample and stored at -20ºC. Two hundred ml deionized water plus 2 g of NaCl was added to each sample and then shaken for 30 minutes at 150 rpm by means of a Gallenkamp shaker. Samples were filtered through Whatman No.1 filter paper, 10 ml of the filtrate was filtered through GF/A glass microfibre filter, then 2 ml of the extract (equivalent to 0.25 g sample) was passed through the DONPREP immunoaffinity column at a flow-rate of about 1 drop min^{-1}. The column was rinsed with 5 ml de-ionized water at the same flow rate; the analyte was eluted with 1.5 ml methanol and collected in a clean 4 ml dark glass vial. The eluant was evaporated under a nitrogen stream at 40ºC and dissolved in 1 ml of the HPLC mobile phase solution (acetonitrile: water, 1: 9) (MacDonald *et al.* 2005).

HPLC Analysis

Chromatographic separation and detection was performed using a Waters 616 pump and a Waters 486 tunable UV absorbance detector set at 218 nm and equipped with an automatic injector (Waters 717). Signals were processed using the Millennium

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software (version 3.2). The reverse phase column was a Waters Nova-pak® C-18, 3.9 mm×250 mm, 4µm particle size (Waters Milford, MA, USA). The mobile phase was acetonitrile: water mixture (1:9; v/v) and was used isocratic at 1.0 ml min^{-1} flow rate. The mobile phase was degassed through a vacuum–degassing device (Waters). The injection volume was 200 µl (equivalent to 0.05 g of sample). The quantification of DON was estimated by integrating the area under the curve for DON at its retention time and was compared to a standard calibration curve for this analyte by using standard, spike and blank samples (Figure 1). DON retention time was 6.7 min under the above conditions.

Recovery Test

To prepare 500 and 1,000 ng g^{-1} spiked corn samples, appropriate amount of

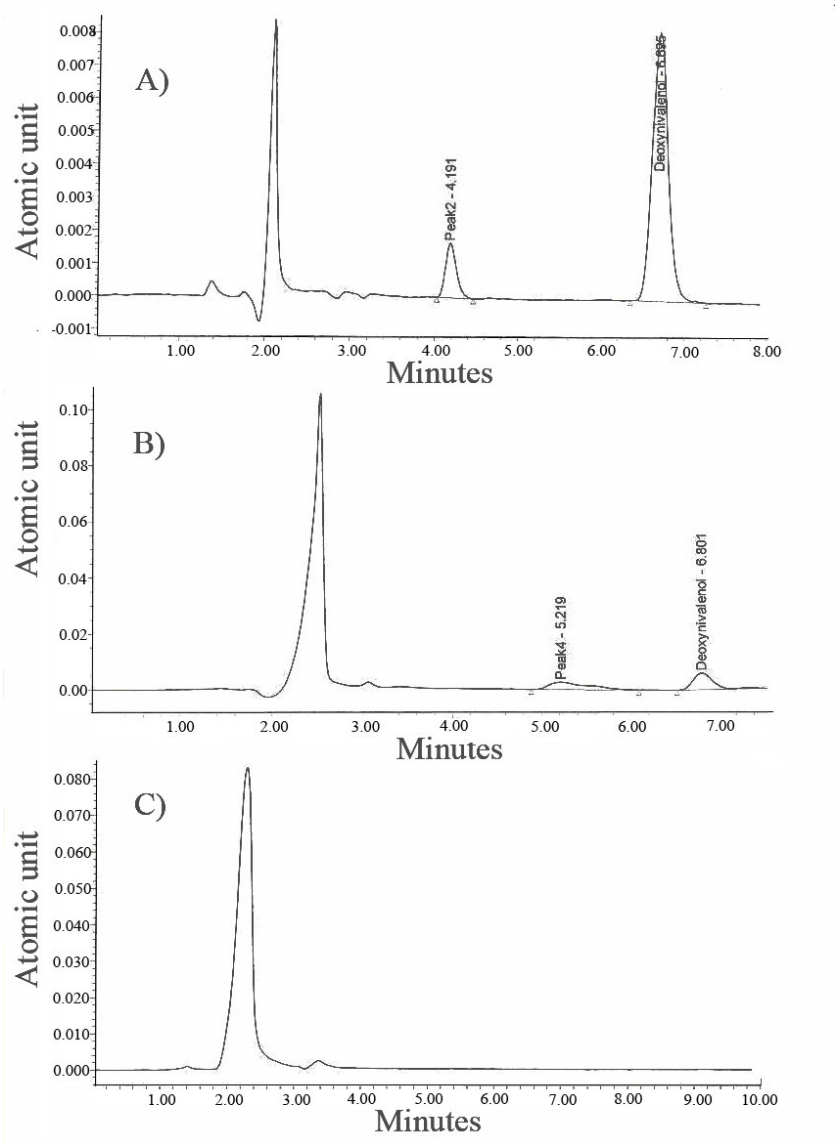

Figure 1. HPLC chromatograms of (A) Deoxynivalenol standard 1000 ng ml^{-1}, (B) Corn spiked sample with 1.0 ppm (C) Blank corn sample. HPLC condition, column: 250×3.9 mm, C18 reverse phase ODS, 4 µm; mobile phase: acetonitrile: water (1:9; v/v); flow rate: 1.0 ml min^{-1}; UV detector: 218 nm.

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standard solution (10 µg ml^{-1}) of DON was added to 25 g of the blank corn samples and left for one hour to have the solvent evaporated prior to extraction. Extraction and clean up were carried out as for the other samples. The detection limit was 10 ng g^{-1}. A positive correlation was detected among all points of the standard curve in the range of 50 to 10,000 ng ml^{-1}; with a linearity of R^2= 0.9999. Mean recoveries for 500 and 1,000 ng g^{-1} spiked sample were 93.5 and 73.5% (n= 5), respectively.

RESULTS AND DISCUSSION

The analysis of 60 corn samples, which were collected during 2004-2005 from Golestan and Moqan areas, revealed that 76.6% of samples were contaminated with DON (Table 1), the range of contamination was 54.4 to 518.4 ng g^{-1} which was under recommended levels for DON in the world. Since 80% of analyzed samples (48 samples) were collected during post harvest stages, a validated statistical regression between contamination levels or contamination incidence percents of samples and sampling stages was not found. The following presented results are the first reported determination of DON in corn from Iran.

Fusarium graminearum benefits from a broad host range and can cause ear disease of corn often called red ear rot or *Gibberella* ear rot. Red ear rot has been reported in corn–growing areas worldwide but is especially prevalent in temperate climates where relatively cool temperatures and weather coincide with silk emergence. Natural epidemics are often localized and sporadic and thus difficult to predict. During early stages of symptom development, infected ears are not easily detected in fields because husks may show few symptoms. In severe infections, however, red or pink mold can progress throughout the entire ear, colonizing kernels, cobs, and husks. If wet weather persists, blue-black perithecia and a pinkish mass of mycelia and macroconidia can form on husk surfaces. Spores landing on the emerged silks can infect the ear through the silk channel. Controlled field inoculation into the silk channel indicates that corn ears are most susceptible to *F. graminearum* within 4 to 7 days after silk emergence (Reid *et al.*, 1992). Wound caused by birds, insects, or extreme weather can provide an opportunity for fungal invasion; such ear damage is positively correlated with ear rot (Sutton 1982; Vigier *et al*., 1997). Ascospores and macroconidia produced by *F. graminearum* growing on corn stalks and other crop residues are the major inocula for both wheat head scab and corn ear rot. In addition, there was a positive correlation observed between *F. graminearum* ear rot and rainfall in July and August when corn is in silking (Vigier *et al*., 1997). Epidemics also appear to be associated with wet weather late in the growing season (Miller, 1994). Studies on corn infected with *F. graminearum* in the field indicate that corn can be contaminated with DON and zearalenone. Corn cob tissue often contains the highest concentrations of DON in the ear (Reid *et al.*, 1996). DON levels in kernels of corn are highly correlated with disease severity ratings and

Table1. Total contaminant samples (%), range and mean of DON contamination (ng/g) for each region at harvest and before silo

Location	Contaminant samples%	DON (ng g^{-1}) range	DON (ng g^{-1}) mean
Golestan (Ali Abbad)	80	57.6-518.4	161.3
Golestan (Aq-Qala)	64.7	54.4-357.3	104.23
Moqan	85.7	61.6-117.2	73.31
Total mean	76.7	54.4-518.4	116.25

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with various measures of fungal load, including colony- forming units and ergosterol levels (Atanassov *et al*., 1994; Bennett *et al*., 1988; Miller *et al*., 1983; Trigo-Stockli *et al*., 1995). However, even visibly healthy grain can contain significant amounts of DON. In an Ontario study, 52% of normal-appearing corn kernels collected from an infected field contained 0.28 to 5µg g^{-1} DON by ELISA (Sinha and Savard, 1997).

In spite of the wide studies conducted on *F. graminearum* as the causal agent of *Fusarium* head blight diseases of wheat and barley throughout wide areas in Iran (Safaie *et al.*, 2005; Safaie and Alizadeh, 2001; Sanjarian *et al*., 2005), it has not yet been isolated from corn. *F. culmorum,* which was known as a DON producer (Birzele, 2002), was isolated from Iranian maize mycoflora (Ghiasian *et al.*, 2004). It seems that the reason for low isolation frequency of *F. graminearum* and *F. culmorum* from corn seed in Iran is due to the dominant population of *F. verticillioides* and *F. proliferatum* among the other *Fusarium* species on maize at harvest time, in which case both compete with *F. graminearum* growth in artificial media in *in vitro* experiments. Moreover fumonisin B1 contamination of the Golestan's corn seed samples which were used in this research and evaluated using HPLC+IAC was quite high as all the samples were contaminated (Mirabolfathy *et al*., 2007) in the range of 261-6,891 ng g^{-1} with a mean of 2,658.35 ng g^{-1}. If selective media had been used for isolating *F. graminearum,* among the other *Fusarium* species, with altering temperature during incubation period and collecting samples at early harvest time, DON producing *Fusarium* species could possibly be more frequently isolated. Since the residues of infected wheat and barley are the main inoculum sources of infection, especially whereby wheat and barley are planted in rotation with corn in a majority of fields in Iran, it seems monitoring of *Fusarium* species during corn growth stages from silking through harvest would help in identifying DON producing *Fusarium* species. A monitoring of *Fusarium graminearum* conidia on corn ears in corn growing fields would help to prevent and allow for inhibition of DON incidence in corn, while employing the integrated pest management principles.

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آلودگی طبیعی محصول ذرت مناطق گلستان و مغان به داکسی نیوالنول

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چکیده

محصول ذرت سالهای ۸۵-۱۳۸۴استان گلستان و مغان از نظر آلودگی به داکسی نیوالنول مورد بررسی قرار گرفت. نمونه ها از مراحل مختلف شامل قبل از برداشت، در مرحله برداشت، پس از برداشت و بعد از خشک نمودن دانه های ذرت جمع آوری گردید. زیر نمونه های آسیاب شده با استفاده از آب عصاره گیری و با ستون های ایمونوافینیتی تصفیه گردید. میزان داکسی نیوالنول نمونه ها با استفاده از کروماتوگرافی مایع فاز معکوس ارزیابی شد. منحنی استاندارد برای محلول های استاندارد در غلظت های ۱۰۰۰۰- ۵۰ نانوگرم در میلی لیتر کاملا خطی بود، حد تشخیص روش ۱۰ نانوگرم درگرم و بازیافت آن برای نمونه های غنی شده حاوی ۵۰۰ و۱۰۰۰ نانوگرم درگرم داکسی نیوالنول به ترتیب ۵/۷۳ و ۹۳/۵ درصد بود. آلودگی به داکسی نیوالنول در ۷۶/۷ نمونه ها مشاهده گردید و گستره آلودگی برابر ۵۴/۴ تا ۵۱۸/۴ نانوگرم درگرم و میانگین آلودگی در نمونه های آلوده ۱۱۶/۲۵ نانوگرم درگرم ارزیابی گردید.

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