Identification of fungi associated with storage corn (zea mays) and estimate fumonisin B1 production by *Fusarium* spp.isolated from these cereals

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Abstract

Zea mays one of the most important grains produced in Babylon province of Iraq incidences of contaminated seeds with toxigenic fungi were investigated in Babylon maize in 2016 from four Locations (centres (of accumelation corn seeds) namely medhateia, Babil, mussaieb and Locally markets, present study included Identificate the mycoflora of maize in these locations. Mycological analyses showed a predominance of *Fusarium*sverticilliodes (48.5%), followed by *Aspergillus* flavus (16.5%), F. proliferatum (6.5%), *Penicillium* species (5%), *Mucor* species (1.43%) in maize

Sixity strains of *Fusarium verticillioides* and *F. proliferatum* isolated from corn collected from fourtht stores in Babylon province, were evaluated for their ability to produce fumonisins B1 (FB1), in Rice culture. Fumonisin levels were determined by high-performance liquid chromatography.All tested strains of *F.verticillioides* and *F. proliferatum* produced fumonisins within a wide range of concentrations,233-1471ppb,176-823ppb,134-792 ppb and 236-1271ppb FB1. The highest mean concentrations of FB1 were 674,617,548and 340 ppb for location (localy markets,medhateia mussaieband and babil, , respectively) from the four locations of province were observed. Fumonisin mean levels produced byF. *Verticillioides* and *F. proliferatum* strains isolated from localy market zone) were significantly ($P < 0.05$) higher than the other three location.

Keyword: *Fusarium*, *Aspergillus penicillium*, Fumonisin, corn

Introduction

spread seed-borne fungi and contaminationby mycotoxin producing species in maize [1], is important in evaluate the risk of mycotoxi n contamination. A number of fungal species related with maize, mainly belonging to the genera*Fusarium* and *Aspergillus*, and peniccillum have been reported to produce mycotoxins which cause mycotoxicoses in humen and animals [2]. In some maize producing countries, information is available regarding the mycoflora of stored corn creals, including Argentina [3]; United States ,South Africa ,Canada [4] and some other countries [5]. The growth of fungi in store is controlled via the following factors such as:(1) composition of nutrients in the seeds(2) humidity and temperature conditions(3) biotic factors as competition or thefounding of stored product or damage of insects.Storage fungi are much more frequent in lots infested via stored product insects, because insects generate humidity and seperate fungi spores in the commodity [6]. Storage fungi require a relative humidity of at least 65%*)* which is equivalent to an equilibrium moisture content of 13% in cereal grain. Theygrow at temperatures of between 10 and 40°C [7].

Mycotoxins are secondary metabolic atoxic substances which are produced by different fungi. They stay in the stored produce as residues. Mycotoxins can be found in the stored products as soon as 24 h after infestation with fungus. The suitable climatic conditions for the growth of fungi and the formation of mycotoxins are almost not corresponding and dependent on divers unidentified factors [8].

All mycotoxins are low-molecular-weight natural products (that is, small molecules) produced as secondary metabolites by filamentous fungi

The fumonisins are a group of structurally associated mycotoxins produced fundamently by *Fusarium verticillioides* (Sacc.) Nirenberg (for *F.moniliforme* Sheldon)and*F. proliferatum*(Matsushima) Nirenberg, both of which frequently infect Maize [1] crops worldwide [9].

Fumonisins were first isolated in 1988 from culturesof *F. verticillioides* MRC 826, which was firstly gained from corn in a high oesophagealcancer (OC) rate area in the Transkei regionof South Africa [10, 11] *F. verticillioides* and *F. proliferatum* have been isolated from corn worldwide global those that appear well these fungi have been recognized as the highest fumonisin producers, and most isolates of these two species are able to produce fumonisin [12].

Inspite of 28 structurally associated fumonisin analogues have been recognised only three, fumonisins B1 (FB1), B2 (FB2) and B3 (FB3) occurat aplenty levels. They are frequently appeared in fungal cultures and/or in naturally contaminated corn, which is an important of ingredient human and animal diets in many countries [13] FB1 generallyrepresents 70–80% of the total fumonisin levels, FB2 accounts for 15–25% and FB3 usually makes up from

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3 to 8% when cultured on corn,rice or in liquid medium [12].

Fumonisins causes many diseases on animal such as leukoencephalomalacia (LEM)in horses [14], edema in pigs and liver cancer in Rats [11] also effect on human studies revealed corelation between consumption diet contaminated with *F.verticilloides or F.proliferatum* With high of OC [12].

The International Agency for Research on Cancer (IARC) in 2002(IARC 2002) classified FB1 as possibly carcinogenic to humans (class 2B carcinogen).

F. verticillioidesand F. proliferatum as the most common seed borne fungi in many country south Africa and in northern Iran.

In present study, corn designed for human and animal consumption in Babylon /Iraq was collected from four main stores of corn creals .

The production of Fumonisins by strains of *F. verticillioides* and *F. proliferatum*, isolated as internal mycoflora from corn seeds, was evaluated. The fumonisin levels produced by these *Fusarium* species were compared between the four stores.

Metrial Methods:

samples of seeds (each ca. 100seed) from each sample were surface sterilized by immersion in(2%)sodium hypochlorite solution in a 100ml Erlenmeyer flask for 1 min, washed twice in steriledistilled water and dried in two layers of sterilised filter papers. (5 per 90 mm plate) on PDA medium containing 500 mg/Lof chloramphenicol to prevent the growth of bacteria [1]. After incubationfor 5–7 days at25_C in the dark the developing fungal colonies were counted directly and where different fungi were isolated from single seed all the colonies were recorded and different species subcultured onto potato dextrose agar(PDA) [15].

Identification of fungi

Identification of *Fusarium* species subcultures were made on PDA incubated for 7 days at 25◦C. Final identifications were made following [4] for [13, 16].

The isolation frequency (Fq), and the incidence of genera and species isolated [17, 18].

were calculated as follows:

Frequency *(%)* = Number of samples in which a genus/species occurred×100/Total number of samples

Incidence *(%)* = Number of grains infected by a genus/species×100/Total number of grains

Ability of Fusarium isolate for production Fumonisin toxin

isolates of *F. verticilliodes and F. proliferatum* were cultured on rice medium [1] for FB1 detection.

Extraction and Cleanup

FB1 was extracted from the finely ground sample using a protocol developed by Maheshwar et al. [19]. 10g samples were placed into a 250 ml conical flask containing 50 ml of acetonitrile: water (ACN: water, 50+50, v/v) and the flask was covered and shaken for 30 min. 15 ml of the supernatant was filtered through a Whatman No. 4 filter paper. A C18 Sep Pak solid phase extraction (SPE) clean up column was preconditioned by rinsing with 2 ml ACN followed by 2 ml of 1% aqueous potassium chloride (KCl). The filtered extract (2 ml) and 1% KCl (6 ml) were mixed in a vial and applied to the column [19].

High performance liquid chromatography (HPLC) analysis involved use of ahplc instrument SYKAM for quantification of fumonisin FB1with

Fluorescnce detector (RF-20A) used for all isolates which be studied.

Results and discussion

Isolation and identification of fungi related corn

A total of 60 fungal isolates were rescued from maize samples collected from the four locations maize Stores in Bbylon provinces of Iraq at 2016. Established onmeans of incidences *Fusarium verticilloides* were the most frequent (48.0%) followed by *Aspergillus flavus* (16.5%), *F.proliferatum* (6.5%),*penicillium*(5%), *Rhizopus* sp.(3.5%) and *Mucor* species (1.43%) (Other genera, were isolated at low frequencies shown in table (1)

Table (1) Incidence and Frequency of fungi related toZea *mays* cereals

%		Isolated fungi
Frequency	Incidence	
1.43*	50	*Alternaria alternata*
17.85	100	*Aspergillus flavus*
5.00	100	*A niger*
1.43	25	*A.oryzae*
2.14	50	*A prasiticus*
1.43	25	*A. terreus*
4.28	75	*Fusarium proliferatum*
3.57	50	*F. solani*
48	100	*F. verticelloides*
4.28	100	*Fusarium* spp
1.43	25	*Mucor* spp
5.00	75	*Penicillium* spp
3.75	50	*Rhizoctonia solani*
3.56	25	*Rhizopus* spp
100 %		Total
Every number represent four replicates. *		

The spread of *F. verticillioides* as a predominant seed-borne fungus in storage seeds of Babylon stores of, Iraq was contrast to the other studies by Aiyaz et al. [20], Moshiri et al. [21] and countrie s such as South Africa [11] United States [22] Brazil [23] with the sutable climatic conditions of this province to this fungi*F. proliferatum* was the second most predominant*Fusarium* species. Several studies on the distribution of *F. proliferatum with F.verticilloides* on corn, rice [24].

HPLC detection of fumonisin in stored corn grains

In the present study ability of producing fumonisin by *Fusarium* strains from four locations was investigated. Detailed results are shown in Table 2 and Fig.1, The 60 strains of *F. verticillioides* and *F.proliferatum* 55strains of *F. verticilloides* 5 strain of *F.proliferatum* all produced FB1

Table (2) Incidence and concentration range of mycotoxins detected by high performance liquid chromatography

Location	Percentage % incidence	Concentration range (ppb)

Medhatia	100	233.34-1471
Babil	100	134.5-792.00
Mussaib	100	176.5-821.00
Local markets	100	236.00-1275

There was a high degree of variability in the concs. of fumonisins produced by *F. verticillioidesand F. proliferatum* In some cases, large differences in fumonisin production can be observed between strains recovered from the same location or from the same store. The *F. verticillioides* culture material had FB1 levels ranging from 233.34–1471ppb ,134–792 ppb, 176.5-. 821 ppb and 236-1275ppb respectively [13, 25].

A further analysis of general mean for the mycotoxins detected by hplc infour location in Babylon provience (Figure 1), shows that there was a spread of FumonIsin (B1) in stored corn cereals

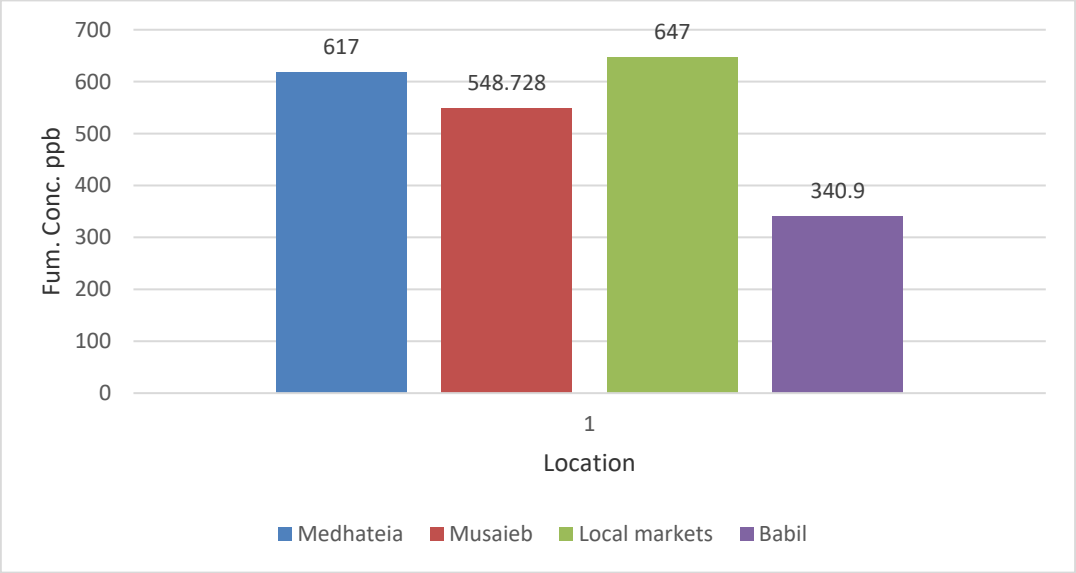

Figure 1. Comparison of Fumonisin contamination between four stores for seed corn

The results explains highest mean levels of FB1were produced from location (locally markets) i,e 674ppb and lowest levels from Babil location i,e340.93 Our results are in acontrast with other reports that many strains of *F. verticillioides* and*F. proliferatum* isolated from corn and other widely differs from different locations that's found in, i.e. North America, Africa ,Asia and Australia, have a high potential for fumonisin production [10, 26]. The production of fumonisins by different strains of *F.verticilloides* varies widely.

Commonly, environment conditions such as high temperatures and moisture, unseasonal rains during harvest, and Insect damages lead to fungal proliferation and production of mycotoxins. Poor harvesting practices, un suitable storage and less optimal conditions during transport and marketing can also engage to fungal growth and increase the risk of mycotoxin production [27].

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