Fungal Genomics & Biology
ISSN: 2165-8056

Almoammar et al., Fungal Genom Biol 2014, 4:1
DOI: 10.4172/2165-8056.1000115

Research Article Open Access

Fungal Infestation and Mycotoxin Contamination in Camel Feedstuffs

Hassan Almoammar[1,2], Ali H Bahkali[2], Mohammad A Khiyami[4] and Kamel A Abd-Elsalam[3,4*]

[1]Botany and Microbiology Department, Faculty of Science, King Saud University, Riyadh, Saudi Arabia
[2]King Abdulaziz City for Science and Technology (KACST), Riyadh Saudi Arabia
[3]Agricultural Research Center, Plant Pathology Research Institute, Giza, Egypt
[4]Unit of Excellence in Nano-Molecular Plant Pathology Research (ARC), Giza, Egypt

Abstract

Currently, mycotoxins and toxigenic fungi feedstuffs are known causes of animal disease. The main aims of the current research were to investigate the mycoflora associated with feed samples consumed in Saudi Arabia and evaluate the occurrence of toxigenic species. Results of mycobiota showed that most samples were contaminated by moulds belonging to the genera, *Alternaria, Aspergillus, Fusarium, Penicillium*, *Setosphaeria*, and *Nuersopra*. In terms of the analyzed mycotoxins, *F. verticillioides* was the highest producer of fumonisin, zearalenone and DON (1050, 245, and 640 µg/kg, respectively. *A. alternata* was the highest producer of Altenuene Alternariol (52, and 29 µg/kg, respectively). Charm ROSA quantitative lateral flow assay method was used to quantify the total aflatoxin. The aflatoxin B1 was detected between 1-3.5 ppb in the collected camel feeds samples, while, feeds samples contained the midst concentration of total aflatoxins. In conclusion, camel feedstuffs can be seriously contaminated by mycotoxigenic fungal genera as well as mycotoxins that cause serious health problems for camel and animals.

Keywords: Toxigenic fungi; Feed; Mycobiota

Introduction

Production of mycotoxins can occur in all agricultural products under suitable field or storage conditions throughout the animal feed supply chain]1]. There are over 100 species of fungi that can infect plants and produce mycotoxins. Mycotoxins are mainly belonging to three fungal genera including; *Aspergillus*, *Penicillum* and *Fusarium*]2]. Mycologist have identified 300 to 400 mycotoxins to date]3], only a very limited number is subject to legal guidance and regular screening. aflatoxins (AF Fumonisins (FB), deoxynivalenol (DON), zearalenone (ZEN) and ochratoxin A (OTA) are most often confirmed for feed products]4]. Mycotoxin-producing Fusaria are disseminated worldwide and feedstuffs contaminated with their toxins have been found in nearly all published surveys]4,5]. The Fusarium fungi are probably the most prevalent toxin-producing fungi, the most common Fusarium toxins detected were zearalenone (ZON), fumonisins (FBs), moniliformin (MON), and deoxynivalenol (DON)] 5]. Because most of these mycotoxins are a potential health risk to farm animals]6,7]. Some countries have regulated mycotoxins in feed at maximum acceptable levels]8]. *Fusarium* mycotoxins were most common, but a number of *Alternaria* toxins also occurred very often]9]. The raw feed components (grains, seeds, and meals) are an optimal media for fungal development due to their high level of nutrients. Certain ecological conditions, mainly the moisture, temperature, pH and light favor fungal growth and development. Infestation may occur in the fields, upon harvesting, during storage and even during processing leading to the loss of nutrients and to the development of toxic compounds known as mycotoxins]10,11]. Since Saudi Arabia has cool, hot and humid type of environment, the chances of occurrence of important and major mycotoxins like aflatoxin, zearalenone, trichothecenes, ochratoxin and fumonosins are more probable in the animal feed. The identification of toxigenic fungi is very important to prevent toxin contamination of animal feed during both pre-harvest and post-harvest. Currently, there is an additional need for these methods to be applied in developing countries to screen large numbers of toxigenic species. The purpose of this study was to survey the stored camel feed in market and to assess the present status of fungal and co-occurrence of mycotoxins in feeds produced and marketed in the country.

Materials and Methods

Feed samples

Twenty samples including 14 samples of natural feed (wheat bran, barley grain, and sorghum), and 6 samples of compound feed (manufactured pellet and premixes) used in camel feeding in Saudi Arabia were collected from various animal feed factories, storehouse and fodder markets from vendor or distributors during 2009. Two sites were selected for the study. The first site was an area south of Riyadh (Wadi-Aldawaser Governorate) and the second was east of Riyadh city (Aziziyah area). Fungal species were isolated from different feeds including wheat bran, barley grain, fresh barely, compound feed, crushed corn, sorghum, and millet grain. The collected feed samples is stored at a moisture content equivalent to 14% for three months.

Fungal isolation

Fifteen pieces from feed samples were used either after being surface-sterilized (using 1% sodium hypochlorite solution and washed three times with sterile distilled water). Ten grains in each case were placed randomly on the surface of Petri-dishes containing Potato Dextrose Agar (PDA) in triplicate. Plated grains were incubated at 25±2 °C. All plates were examined after 3, 5 and 7 days of incubation, using a stereomicroscope (Nikon SMZ-U), to accompany fungal growth. After 7 days of incubation, all fungi belonging to genus Aspergillus and some fungi representative of other genera were transferred into 9 cm Petri

***Corresponding author:** Kamel Abd-Elsalam, Plant Pathology Research Institute, Agricultural Research Center (ARC), Egypt, Tel: 002 01091049161; E-mail: kamelabdelsalam@gmail.com

Received November 30, 2014; **Accepted** December 04, 2014; **Published** December 11, 2014

Citation: Almoammar H, Bahkali AH, Khiyami MA, Abd-Elsalam KA (2014) Fungal Infestation and Mycotoxin Contamination in Camel Feedstuffs. Fungal Genom Biol 4: 115. doi:10.4172/2165-8056.1000115

dishes containing 15 mL of PDA with an inoculation needle previously examined daily for five days, after which the colonies developing from the grains were counted. Isolated fungi were purified either by single spore or hyphal tip methods, and then transferred to slanted PDA. Isolates identification was carried out based on morphological and microscopic characteristics in the Mycological Center, Assiut University, Egypt.

Fusarium mycotoxins

Fusarium mycotoxins (Fumonisin, HT-2, Zearalenone, T-2, Neosolaniol and DON) content was determined using the VICAM (1998) assay]12]. The method was similar with all former toxins except the dilution buffer, developer and immune-affinity column. Each isolate was grown in Erlenmeyer flask 100 ml on SMKY media. The incubation period was 7 days at 25±2°C. After blending on high speed for 1 min. with 5 g of sodium chloride. 20 ml of culture filtrate was added to 80 ml of methanol (HPLC grad) and filtered through a fluted filter paper. The extract (10 ml) was diluted with 40 ml of phosphate buffered saline (PBS) 0.1% Tween-20 wash buffer and filtered through a 1.0-Am microfiber filter. The diluted extract was passed through the column, which was washed with 10 ml of PBS/0.1% Tween-20 wash buffer followed by 10 ml of PBS. Fumonisins were eluted from the column with 1 ml HPLC grade methanol. A mixture of developer A and developer B (1 ml) was added to the elute collected in a cuvette that was placed in a fluorometer (VICAM Fluorometer Series 4, Watertown, USA) for fumonisin measurement.

Alternaria mycotoxins

Flasks were made up containing 12.5 g of autoclaved polished rice at 40% moisture. Flasks were inoculated with agar plugs of one-week-old cultures of *Alternaria* spp. isolate. The flasks were incubated in the dark at 25 °C for 21 days]13]. The method for the detection of *Alternaria* toxins in rice was described by]13]. The culture material was homogenized with 30 ml of methanol and filtered through a Whatman filter paper (no. 1). The filtrate was clarified with 60 ml of 20% ammonium sulphate. Culture filtrate was extracted three times with 10 ml of chloroform. The organic phases were combined, evaporated to dryness, and dissolved in 4 ml of methanol for AE, and AOH analysis by high-performance liquid chromatography (HPLC).

HPLC detection

The HPLC system consisted of a Shimadzu liquid chromatography (Shimadzu, Kyoto, Japan) equipped with a Shimadzu SPD-M10Avp UV photodiode array detector. The analytical column was ODS 4.6x250 mm 5 u. C18. Standards of AE and AOH were purchased from SIGMA Chemical Company (St. Louis, MO, USA). The mobile phase was methanol/water (80:20) containing 300 mg ZnSO4-H2O/l, for AE, AOH and AME. A flow rate of 0.7 ml/min was used. The wavelength for recording chromatograms was 258 nm for AE and AOH According to]14].

Aflatoxin bioassay using *Abelmoschus esculentus* seedling

A. flavus strains were inoculated on potato dextrose broth (PDB) and incubated for 2-4 weeks and spores were harvested as formerly described by]15]. Okra (*Abelmoschus esculentus*) seedlings were inoculated by placing 10 µl of *Apergillius* species conidial suspension (containing 10^6 conidia) on each emerging seedling leaf. Two leaves per seedling were inoculated. Control plants were treated with 10 µl of sterile distilled water. Inoculated plants were incubated in a moist chamber for 72 h and returned to the growth chamber at 21°C (day)/16°C (night) temperatures with day/night regime of 16h/8h.

Quantitative lateral flow assay method for aflatoxins in feed samples

The Charm ROSA Aflatoxin (quantitative) kit was calibrated using either a 70% methanol extraction or a 50% ethanol extraction. The procedure is: the milled/ground sample was extracted with solvent, 100 µL of extract was added to 1 mL dilution buffer, 300 µL of the was diluted extract is added to a lateral flow test strip. The test strip was incubated for 10 minutes at 45°C. The test strip was removed and read in the calibrated reader. The ROSA-M reader provides a numerical result from 0 to 250 ppb by comparing the binding intensity of 2 test lines and a control line. Corn, wheat, cracked corn, popcorn, soybeans and rice were tested with this kit using both solvent extraction methods.

Results

Identification of fungal isolates

Seven different species of fungi were identified. The genera Aspergillius (52.2%) and Alternaria (15%) and Fusarium (12.5%) showed the highest prevalence in the samples investigated. A. flavus and A. niger were found to be the most prevalent fungal contaminants in feed products collected from Riyadh region (Figure 1). A polyphasic method for was used to identify aflatoxigenicity of aspergilli isolated from feeds for the same isolates associated in the current study by Almoammar et al.]17].

Screening of Fusarium for mycotoxin production

All isolates were toxin producers. Toxin-producing isolates diverse in the type and concentrations of toxin produced. *F. verticillioides* was the highest producer of fumonisin, zearalenone and DON (1050, 245, and 640 µg/kg, respectively). *F. semitectum* isolate number 1 was the highest producer of fumonisin, zearalenone, DON, and HT-2 (850, 245, 85, 550 and 0.9 µg/kg, respectively) (Table 1).

Screening of Alternaria for mycotoxin production

Toxin-producing isolates varied in the type and concentrations of toxin produced. All *Alternaria alternate* isolates was able to produce Altenuene and Alternariol toxin (Table 2). *A. alternata* was the highest producer of Altenuene and Alternariol (52, and 29 µg/kg, respectively).

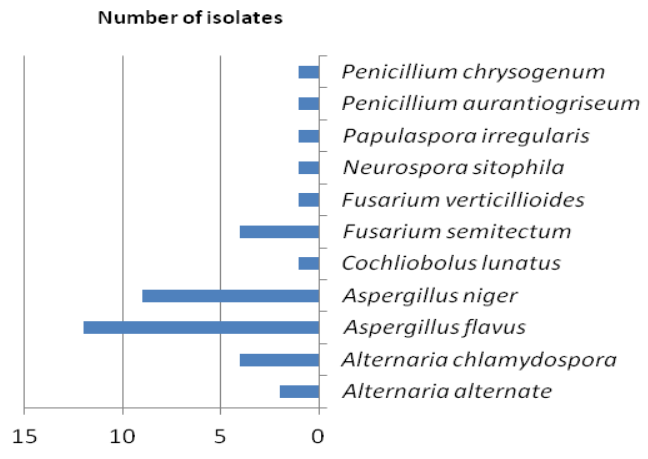

Figure 1: Identified fungal species isolates analyzed in this study.

Isolates	Mycotoxins (µg/kg)				
	Fumonisin	Zearalenone	T-2	DON	HT-2
F. semitectum	850	85	2.0	550	0.9
F. semitectum	700	50	4.0	320	1.0
F. semitectum	450	60	2.0	120	3.5
F. semitectum	320	130	7.0	210	0.0
F. verticillioides	1050	245	8.0	640	3.0

Table 1: Production of mycotoxin by *Fusarium* spp. isolated from feed products.

Isolates	Mycotoxins (µg/kg)	
	Altenuene	Alternariol
Alternaria alternata	52	29
Alternaria alternata	44	25
Alternaria alternata	23	14
Alternaria chlamydospora	0	0
Alternaria chlamydospora	0	0
Alternaria chlamydospora	0	0
Alternaria chlamydospora	0	0

Table 2: Production of mycotoxin by *Alternaria* spp. isolated from feed products.

Screening of *Penicillium* species and other fungal species for mycotoxin production

Penicillium aurantiogriseum isolate was produced 14 and 30 µg/kg of Penicillic acid and patulin citreoviridin, respectively. *Penicillium chrysogenum* isolate was produced 7 and 12 µg/kg Citreoviridin and Citrinin (Table 3). Isolates varied in the type and concentration of toxins produced. None of other fungal species included *Cochliobolus lunatus*, *Neurospora sitophila*, *Papulaspora irregularis*, *Setosphaeria rostrata* and *Setosphaeria rostrata* was able to produce mycotoxin (Figure 1).

Aflatoxin level of different type of feeds

A total of 20 market samples comprising six different feed commodities from Riyadh region were examined in order to isolate aflatoxin-producing fungi as well as to assess aflatoxins in the commodities. All tested samples were contaminated with total aflatoxin with concentrations ranging from 1 to 3.2 ppb (Table 4).

Aflatoxin bioassay using okra seedlings

Aflatoxin is a naturally occurring toxic chemical by-product from the fungal growth of the *A. flavus* on infected crops. The filtration of liquid culture media for *Aspergillius* isolates was used to screen aflatoxin producing fungi. Young leaves was affected first and become small and distorted or chlorotic with irregular margins, spotting or necrotic areas. The initial symptoms were the yellowing of the entire leaf including veins usually starting with the younger leaves. Leaf tips may yellow and curl downward. Leaf size was reduced and overall growth will be stunted. Leaves yellowing or scorched at edges (Figure 2). Seedling length and total chlorophyll content reduced in aflatoxin treated seedlings.

Discussion

Mycotoxins may be existing in feed products without any visible symptoms of mould infestation. There is a need for rapid and accurate measurement of mycotoxins for purposes of continual screening and identification of high risk commodities]16]. Twenty samples of camel feed used in animal feeding in Saudi Arabia were investigated for their contamination with fungi and/or their mycotoxins. In the current study, the mycobiota of twenty feed samples were surveyed in two cites in Riyadh region. Analysis of fungal contamination of feed products in the present study yielded 13 fungal species belonging to 7 genera. Other potential mycotoxin producers among isolates were *Fusarium, Penicillium* and *Alternaria* while, the lowest fungal species contaminating compound feed were *Penicillium* spp. Other isolated fungal genera including *Helminthosporium* spp. had a very low percentage of occurrences, or were totally missing in samples. All tested isolates were toxin producers. Toxin-producing isolates varied in the type and concentrations of toxin produced. *F. verticillioides* was the highest producer of fumonisin, zearalenone and DON. The very high levels of *F. verticillioides* and *F. semitectum* in feed samples is known to infect crops in the field and propagate at moisture contents. The in vitro production of the toxins deoxynivalenol, zearalenone fumonisin, T-2, and HT-2 was quantitvely evaluated in 8 different isolates of *Fusarium* species collected from feed samples. It was possible to detect zearalenone and the other mycotoxins in 100% and 50% of the isolates, respectively]17]. Lateral flow immunoassay (LFIA) or Gold-colloid-based immunoassay, to produce fully-portable devices, which require no laboratory equipment, minimum skilled personnel, minimum sample preparation, and no hazardous chemicals]18]. The assay can be typically concluded in few minutes and results can be both visually estimated or read by an appropriate reader. A marketable LFIA for the quantitative detection of Aflatoxin (quantitative) Test kit in feeds is available (Rosa Aflatoxin, Charm) and has been validated in an inter laboratory trial, confirming its reliability in the 0-250 ppb range. All tested samples were contaminated with total aflatoxin with concentrations ranging from 1 to 3.2 ppb.

Isolates	Mycotoxins (µg/kg)			
	Penicillic acid	Citreoviridin	Citrinin	Patulin
Penicillium aurantiogriseum	14	4	10	30
Penicillium chrysogenum	11	7	12	25

Table 3: Production of mycotoxin by *Penicillium* spp. isolated from feed products.

Sample code	Feed type	Production year	Total aflatoxin
w-3	Barley grain	2009	2.86
w-4	Wheat bran	2009	1.50
w-5	Millet Grain	2009	1.71
w-6	Compound feed	2009	3.22
w-7	Sorghum	2009	1.00
w-8	Crushed corn	2009	1.84
w-9	Compound feed	2009	2.00
w-10	Compound feed	2009	3.22

Table 4: Amount of aflatoxins (ppb) in different feed samples collected from Riyadh region.

Figure 2: Aflatoxin bioassay using okra seedlings, the leaves infected with *A. flavus* isolates have abnormal colors.

Alternaria species are well known to contaminate a wide variety of crops in the field and to cause post-harvest decay of various grains. In addition, several toxic compounds belonging to different structural groups have been documented to produce by *Alternaria* spp. in cereals]13,19,20,21]. In the current work, all *Alternaria alternate* isolates was able to produce Altenuene and Alternariol toxin. The presence of aflatoxin vital in Aspergillius culture filtrate was evaluated to inhibition of chlorophyll formation in okra seedlings. Okra seedling length reduced in treated seedlings as compared to non-treated seedlings. Maize seeds were found to be very sensitive to aflatoxin. The germinability of seeds was highly reduced when treated with aflatoxin. Seedling length and total chlorophyll content reduced in aflatoxin treated seeds]22, 23]. However, in case has *A. niger* been proven to produce aflatoxins. Two reports]17,24] that some *A. niger* cultures produced aflatoxin B1 have been proved. As found, most isolates were capable of producing detectable levels of both B and G type's aflatoxins (AFs) and maltoryzine, although 4 of the 7 *A. niger* isolates failed to produce any detectable amount of AFs. Some feed samples had exceeded amount of AFT, OTA and ZON and may be contaminated with other mycotoxins which mean implication of fungi in camel health problems and death in Saudi Arabia]25].

Conclusion

feed samples were contaminated with fungi and some toxigenic isolates including *F. semitectum, F. verticillioides*, *Alternaria alternate, Penicillium aurantiogriseum, Penicillium chrysogenum* and *Aspergillius flavus* which were responsible about mycotoxin production. *F. verticillioides* was the highest producer of fumonisin, zearalenone and DON toxins. Compound feed and barley grain feed were more contaminated than sorghum feed which was under the regulatory limit. Okra seedling length reduced in treated seedlings compared with non-treated seedlings. The screened toxigenic fungi are capable of reducing the nutritional values of camel feedstuff

Acknowledgments

This project was supported by King Saud University, Deanship of scientific research, College of Science Research Centre. Also, this work was partially funded by the Science and Technology Development Fund (STDF), Egypt, (STDF), Egypt, (STDF-STF program, Grant no. 4552 to Kamel Abd-Elsalam).

References

1. Bryden WL (2012) Mycotoxin contamination of the feed supply chain: Implications for animal productivity and feed security. Anim Feed Sci Tech 173: 134-158.
2. Akande KE, Abubakar MM, Adegbola TA, Bogoro SE (2006) Nutritional and health implications of mycotoxin in animal feed. Pakistan J Nutr 5: 398-403.
3. Kabak B, Dobson AD, Var I (2006) Strategies to prevent mycotoxin contamination of food and animal feed: a review. Crit Rev Food Sci Nutr 46: 593-619.
4. Binder EM (2007) Managing the risk of mycotoxins in modern feed production. Anim Feed Sci Tech 133: 149-166.
5. Placinta CM, D'Mello, JPF, Macdonald, AMC (1999) A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins. Anim Feed Sci Tech 78: 21-37.
6. Morgavi DP, Riley RT (2007) An historical overview of field disease outbreaks known or suspected to be caused by consumption of feeds contaminated with Fusarium toxins. Anim Feed Sci Tech 137: 201-212.
7. Pestka JJ (2007) Deoxynivalenol: toxicity, mechanisms and health risks. Anim Feed Sci Tech 137: 283-298.
8. van Egmond HP (2004) Natural toxins: risks, regulations and the analytical situation in Europe. Anal Bioanal Chem 378: 1152-1160.
9. Streit E, Schwab C, Sulyok M, Naehrer K, Krska R, et al. (2013) Multi-mycotoxin screening reveals the occurrence of 139 different secondary metabolites in feed and feed ingredients. Toxins (Basel) 5: 504-523.
10. Pitt JI (2000) Toxigenic fungi and mycotoxins. Br Med Bull 56: 184-192.
11. Yaling W, Tongjie C, Guozhong L, Chunsan Q, Huiyong D, Meiling Y, Bert-Andree Z, Gerd S (2008) Simultaneous detection of airborne aflatoxin, ochratoxin and zearlaenone in poultry house by immune affinity column and high performance liquid chromatography. Environ Res 107: 139-144.
12. VICAM. (1998). FumoniTest TM Instruction Manual VICAM, Watertown.
13. Li FQ, Toyazaki N, Yoshizawa T (2001) Production of alternaria mycotoxins by Alternaria alternata isolated from weather-damaged wheat. J Food Prot 64: 567-571.
14. Scott PM, Kanhere SR (2001) Chromatographic method for Alternaria toxins in apple juice. Methods Mol Biol 157: 225-234.
15. Hilton AJ, Jenkinson P, Hollins TW, Parry DW (1999) Relationship between cultivar height and severity of Fusarium ear blight in wheat. Plant Pathol 48:202-208.
16. Naicker D, Marais GJ, van den Berg H, Masango MG (2007) Some fungi, zearalenone and other mycotoxins in chicken rations, stock feedstuffs, lucerne and pasture grasses in the communal farming area of Rhenosterkop in South Africa. J S Afr Vet Assoc 78: 69-74.
17. Almoammar H, Bahkali AH, Abd-Elsalam KA (2013) A polyphasic method for the identification aflatoxigenicity of aspergilli isolated from feeds. Aust J Crop Sci 7: 1707-1713.
18. Krska R, Molinelli A (2009) Rapid test strips for analysis of mycotoxins in food and feed. Anal Bioanal Chem 393: 67-71.
19. Duke SO1, Dayan FE (2011) Modes of action of microbially-produced phytotoxins. Toxins (Basel) 3: 1038-1064.
20. Bräse S, Encinas A, Keck J, Nising CF (2009) Chemistry and biology of mycotoxins and related fungal metabolites. Chem Rev 109: 3903-3990.
21. Scott PM (2001) Analysis of agricultural commodities and foods for Alternaria mycotoxins. J AOAC Int 84: 1809-1817.
22. Shirurkar DD, Wahegaonkar NK (2012) Effect of aflatoxin on germination and seedling growth. Arch Appl Sci Res 4 :2441-2446.
23. Al-Julaifi MZ, Khalil A (1992) Incidence of mold growth and aflatoxin production in locally grown barley used as ingredient for poultry feeds. Arab Gulf J. Sci Res 10: 87-97.
24. Yassin MA, Elsmawaty AMA, Moslem MA, Bahkali AH, Abd-Elsalam KA (2011) Fungal biota and occurrence of aflatoxigenic Aspergillus associated with postharvest corn grains. Fresenius Environ Bull 20: 903-909.
25. Bokhari FM (2010) Implications of fungal infections and mycotoxins in camel diseases in Saudi Arabia. Saudi J Biol Sci 17: 73-81.