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# Study on toxigenic fungi in ruminant feeds under desert conditions with special references to its biological control

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ABSTRACT

A total of 435 samples from feedstuff (130 samples of commercial ration feed storage from 1–30 days, 77 samples of commercial ration stored higher than 30 days, 57 samples from each of, derris, grind mixture, Tibn and wheat bran) were collected from the feed store houses of private farms located at the desert regions of Ras Sudr at South Sinai and Elameria area at Alexandria Governorate, from December 2012 to May 2014. The collected samples were analyzed for fungal growth. The results revealed that, the main moulds observed in the ruminant feeds were *Penicillium* spp., *Aspergillus (A) flavus*, *Cladosporium* spp., *Mucor* spp., *Trichoderma* spp., *A. niger*, *Alternaria* spp., *Rhizopus* spp., *Fusarium* spp., *A. fumigates* and *A. terreus*. In addition, the winter season was of higher incidence for moulds isolation than summer season. The most toxigenic aflatoxins secreted by *Aspergillus flavus* include Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2. The results of biological treatment of Aflatoxins using *Saccharomyces (S) cerevisiae*, showed that, the addition of *Saccharomyces cerevisiae* at a level of $(1 \times 10^6)$ cfu.ml$^{-1}$ and $(1 \times 10^9)$ cfu.ml$^{-1}$ decreased the level of concentration of aflatoxin B1, B2, G1 and G2 and the level of $(1 \times 10^9)$ cfu.ml$^{-1}$ was more efficient in reducing aflatoxins than the lower concentration.



## 1. Introduction

The desert conditions characterized by high temperature with high relative humidity and dry weather conditions. The desert constituted about 95% of the area of Egypt, and this percentage is a large area in relation to the Egyptian land. The main animals breed in this area are the ruminants including cattle, sheep and goats in addition to camels (FAO, 2011). The main causes of food deterioration is the growth of the toxigenic

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fungi that makes the feed unfit for animal consumption in addition to the secretion of some mycotoxins that causing harmful to the animals and its products as well as human health (García-Cela et al., 2012, Armando et al., 2013).

Mycotoxins are secondary metabolites produced by fungi especially those belonging to the genus Aspergillus, Penicillium and Fusarium (Oswald et al., 2012). The main mycotoxins secreted by toxigenic fungi in ruminant feeds includes Aflatoxin (Razzaghi-Abyaneh, 2013); Ochratoxin, Zearalenone, Trichothecenes and Fumonisins (SCF 2000, JEFCA 2001 and Meyer et al., 2003). Aflatoxins had a particular focus of research studies. They are produced primarily by *A. flavus*, *A. parasiticus*, and *A. nomius*, leading to contamination of many commodities used for human food and animals feed (Jouany and Yiannikouris, 2002). Aflatoxins have carcinogenic and mutagenic in human and animals (Barkai-Golan, 2008).

In ruminants, acute aflatoxicosis results in death while chronic aflatoxicosis results in cancer, immune suppression, and other slow pathological conditions. The liver is the primary target organ which results in liver damage. There are substantial differences in species susceptibility (Zain, 2011). Meanwhile, aflatoxin B1 is considered the strongest hepatocarcinogen agent (Hwang and Lee, 2006). The main techniques used for controlling mycotoxins in animal feed includes four different control strategies may be distinguished: (Åberg et al., 2004) the use of high quality and selected feedstuffs. (García-Cela et al., 2012) Control by monitoring for selected mycotoxins in all feed ingredients or final feed independent of quality or origin (Arranz EBurdanpal et al., 2006). Combining feedstuff of normal or high quality with feedstuff of known low cost and quality or high risk of mycotoxin contamination (Armando et al., 2011). Prevention of uptake of mycotoxins by use of feed additives (Lizárraga-Paulín et al., 2013).

Although there are ways to prevent fungal growth and aflatoxins formation, so far no way has been achieved to control mycotoxins in food (Bley N'Dede et al., 2009). Biological control is a promising approach for reducing both preharvest and post harvest aflatoxins contamination. Reductions in aflatoxins contamination with the use of non-toxigenic strains of fungi, has also been demonstrated in corn and cottonseed (Lizárraga-Paulín et al., 2013) and in bread (Milani et al., 2014).The main fungi used for biological control of moulds and fungi in animal feed were *saccharomyces* spp. *Saccharomyces cerevisiae* strains as biological agents were especially used as post harvest diseases control (Francés et al., 2006). Yeasts may act as antagonistic microorganisms thereby considerably decreasing the growth of filamentous spoilage fungi and also ochratoxigenic microorganisms, these strains were previously proposed to be used in silage (Armando et al., 2011, 2012, 2013). This study aimed to isolation and identification of fungi recoverd from feed of ruminants under desert conditions, detection of toxigenic strains of *A. flavus* and measurement of its toxins besides, detoxification of feeds using biological control.

## 2. Materials and methods

**1-Feedstuff samples:** The samples collected from the feed store houses of private farms located at the desert regions of Ras Sudr at South Sinai and Elameria area at Alexandria Governorate from December 2012 to May 2014. Forty hundred and thirty five samples of feedstuffs (130 samples of commercial ration storage from 1–30 days, 77 samples of commercial ration storage higher than 30 days, 57 samples from each of derris, grind mixture, tibn and wheat bran) each sample was placed in a sterile plastic bag then send to the laboratory for screening and identification of fungal growth.

**2-Isolation of moulds**: 225 ml of sterile peptone water were added to 25 g of the feed sample, and thoroughly mixed using homogenizer at 1400 rpm for 2.5 min, and then 1 ml of the dilution was poured into Sabouraud's dextrose agar. Inoculated plates were incubated at 25 °C for one week. At the end of the week, the resulted fungal colonies were separately noticed by the naked eye, during the incubation period, the plates were examined daily for star-shaped mold growth which was picked up under aseptic conditions with its surrounding cultivated medium and transferred into malt extract agar slope then kept at 22–25 °C for further identification.

**3-Identification of the isolated moulds**: Identification of mould genera and species depending on macroscopical and microscopical investigations was carried out according to Fennel and Raper (1965) and Samson (1979) for genus Aspergillus, Arx et al. (1967); Raper and Thom (1969); Samson et al. (1976) and Zycha et al. (1969) for the other mold genera.

**4-Toxigenicity test for *Aspergillus flavus* strains**: The test was done recommended by (Davis et al., 1966) aflatoxins were produced from *A. flavus* which was grown in a liquid medium (yeast extract sucrose broth) (YES) (2% yeast extract-20% sucrose medium) and the inoculated flasks were incubated at 25 °C for 7–10 days then were observed for fluorescence under long-wave UV light (365 nm).

5-**Qualitative estimation of aflatoxins**: according to (Scott and Lawrence, 1995).

6-**Clean-up procedures for high-performance liquid chromatography (HPLC) analysis**: According to (Bragulat et al., 2001).

**7-Quantitative estimation of aflatoxins** by High performance liquid chromatography (HPLC) used for aflatoxins determination was an Agilent 1100 HPLC system, Agilen Technologies, Waldbronn, Germany, equipped with quaternary pump model G 1311A, UV detector (Model G 1314A)set at 254 nm wavelength, auto sampler (model G1329A VP-ODS) and Shim pack (150 × 4.6 mm) column (Shimadzu, Kyoto, Japan) (Romagnoli et al., 2007).

**8-Biological treatment of mycotoxins:** The isolates of toxginic *A. flavus* that showed ability to produce aflatoxins in high concentrations were used for the investigation. Aflatoxigenic nature of isolates was maintained on Sabouraud Maltose Agar (Merck, Germany) at 4 °C. Manually prepared yeast extract-sucrose (YES) broth served as aflatoxin production medium.

**8.1-Baker's Yeast** The commercial baker's yeast (*S. cerevisiae*) collected from different shops was activated in 100 ml of Sabouraud maltose broth. After the incubation period (48 h, 25 °C). Working concentrations of yeast cells suspension in sterile physiological saline were $10^6$ cfu.ml$^{-1}$ and $10^9$ cfu.ml$^{-1}$ per one ml. Influence of two concentrations of yeast cells suspension on the isolates of *A. flavus* was investigated. The controls without adding of baker's yeast were also prepared.