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Occurrence of Aflatoxins in Maize Seed under Different Conditions

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ABSTRACT

This study was conducted to assess the level of aflatoxins in maize under dissimilar conditions. For this purpose, seed with three physical standards (intact, 5% damaged & 10% damaged) was stored in cold, ware-house and metal-bin conditions for 365 days. After storage period, seed samples were quantitatively analyzed by thin layer chromatography (TLC) followed by high performance liquid chromatography (HPLC) for aflatoxins (B_1, B_2, G_1 & G_2). High incidence of aflatoxins as well as *Aspergillus flavus* was observed in ware-house followed by metal-bin storage and cold storage, respectively. Highest levels of total aflatoxins up to 10.50 and 8.60 ppb were recorded in ware-house stored seeds followed by 4.43 and 3.47 ppb in metal-bin stored maize samples. However, in cold storage three samples exhibited aflatoxins production with low level records up to 1.69 ppb in total. Moreover, physical grade was also observed an influential factor for incidence of aflatoxins. Maize with 10% and 5% damage was found with high aflatoxins as compared to intact seeds. *A. parasiticus* was not observed in any treatment during this study.

Key Words: *Aspergillus flavus*; Chromatography; Conservation; Hazardous; Mycotoxins

INTRODUCTION

Production of mycotoxins is a highly critical feature associated with maize seed. It is hazardous for animals as well as human beings (Miller, 1991). Aflatoxins are a major group of mycotoxins produced by some strains of fungi viz. *Aspergillus flavus* and *A. parasiticus* (Nepote *et al.*, 1997; Medina-Martinez and Martinez, 2000). Types of aflatoxins produced by these species are B_1, B_2, G_1 and G_2. *A. parasiticus* strains produce B1, B_2, G_1 and G_2 (Egel *et al*., 1994), whereas *A. flavus* produces $B_1$and B_2, in general (Cotty & Cardwell, 1999). In USA, South America and Mexico, both species infect maize seeds but *A. parasiticus* is uncommon, as compared to *A. flavus* (Payne, 1992). In Asia *A. parasiticus* does not appear to prevail (Siriacha *et al.*, 1991).

Aflatoxins are primarily a problem associated with maize, as it is infested with *A. flavus* and *A. parasiticus* in the field as well as storage (Nasir & Jolley, 2002).

It was reported that stored maize seed exhibited a high incidence of aflatoxins (Sumbali, 2001). Aflatoxins production by the fungi on seeds depends upon the storage conditions. Major factor reported for aflatoxins production in seeds include moisture content (Manoch *et al*., 1988), relative humidity and temperature in storage (Moreno & Kang, 1999), storage period (Liu & Yu, 2006) and storage types (Roy & Chourasia, 2001).

World-wide distribution of aflatoxins shows that aflatoxin contamination generally occurs in the tropical and sub-tropical regions of the world. In Pakistan, maize was found to be more susceptible for aflatoxins production as compared to canola, soybean and rapeseed, respectively (Firdous, 2003). Notwithstanding a lot of work has been done to find association of seed storage conditions for aflatoxins worldwide, nonetheless research has highlighted the phenomenon in agro-climatic conditions of Pakistan.

The present studies were conducted to find intensity of aflatoxins as well as fungal species *A. flavus* and *A. parasiticus*, responsible for aflotoxins production in maize seed under different storage conditions in Pakistan.

MATERIALS AND METHODS

Maize seed: Healthy maize seeds of a popular hybrid M-6525 were collected from local market of grain market Lahore. Each sample was comprised of 1 kg seed with four replications, where intact 0%, while second 5% and third was 10% damaged. The required damaged percentage was attained by puncturing of seeds with sterilized needle. All seed samples were derived from same batch of grain and target samples packed in cotton bags were stored for 365 days under three storage conditions. The 12 treatments were

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placed under three storage conditions.

Storage conditions: Cold (controlled) storage: 7±2°C temperature and 20±5% relative humidity.

a. Ware-house storage: prevailing temperature and humidity, recorded periodically.

b. Metal-bin storage: prevailing temperature and humidity, recorded periodically.

Fungal incidence: *A. flavus* and *A. parasiticus* associated with the maize seed lot was analyzed using the standard techniques of ISTA (2006).

Aflatoxins evaluation: Maize seed samples were ground and 50 g working sample of each finely ground seed sample was used. For defattening, samples were placed in extraction tubes with the fat solvent (hexane) in boiling flasks of a Soxhlet apparatus. The apparatus was run for 24 hours. The defatted samples were oven dried for further analysis.

Detection and quantification of aflatoxins: Each sample was analyzed by thin layer chromatography (TLC) as per procedure of AOAC (2005). The samples exhibiting aflatoxins through TLC were further subjected to HPLC for confirmation of results. The results were expressed in units of ppb (parts per billion).

RESULTS AND DISCUSSION

Data revealed storage conditions as the major factor affecting production of aflatoxins in maize seeds (Fig. 1). Highest levels of total aflatoxins up to 10.5 and 8.6 ppb were recorded in ware-house stored seeds followed by 4.43 and 3.47 ppb in metal-bin stored maize samples. In cold storage three samples exhibited aflatoxins production with low level records up to 1.69 ppb in total. Physical grade was

Fig. 1: Aflatoxins B_1, B_2, G_1 and G_2 in maize seed under cold, ware-house and metal-bin storage conditions
A1 to A4: intact seed; B1 to B4: 5% damaged seed; C1 to C4: 10% damaged seed

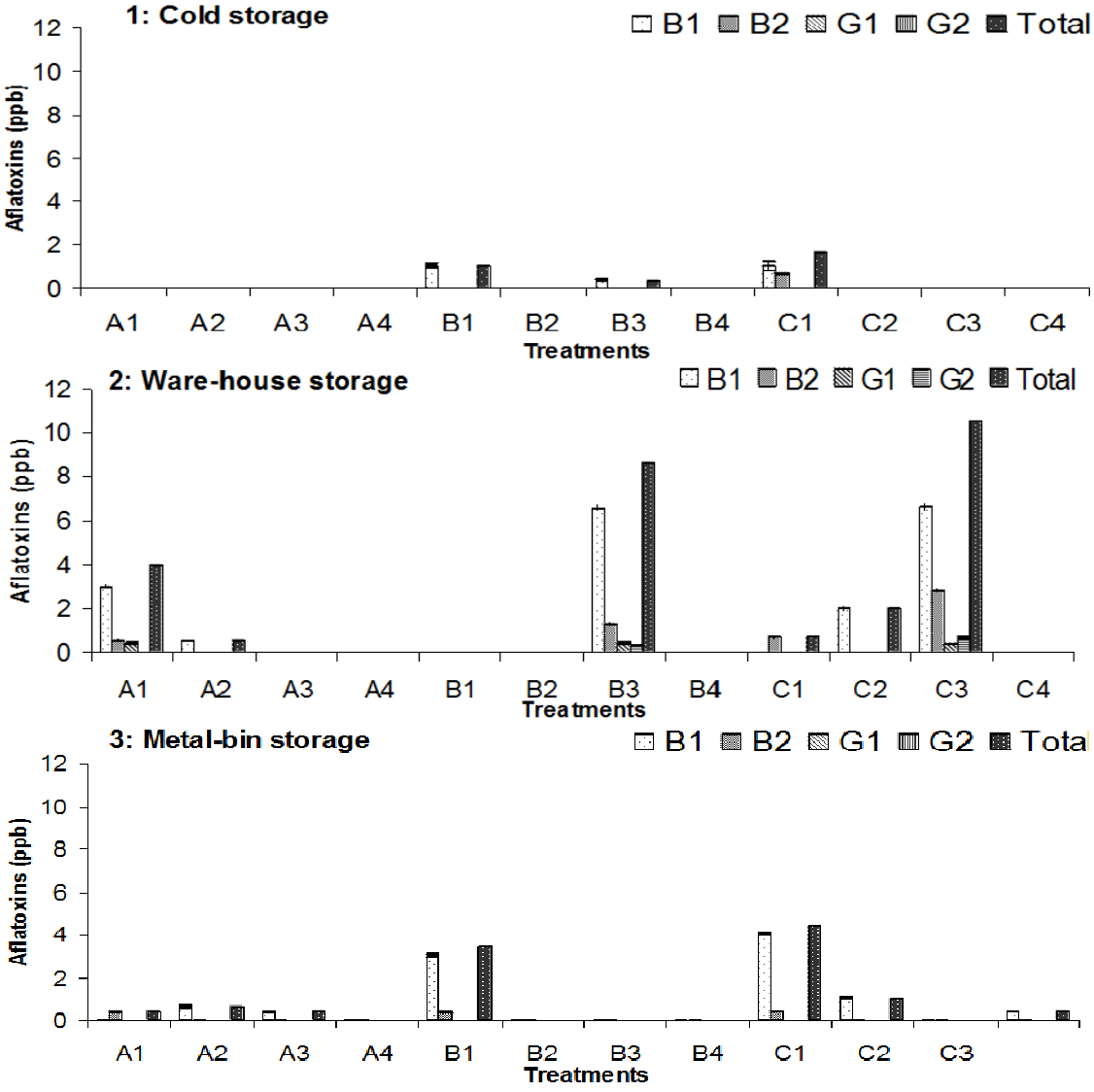
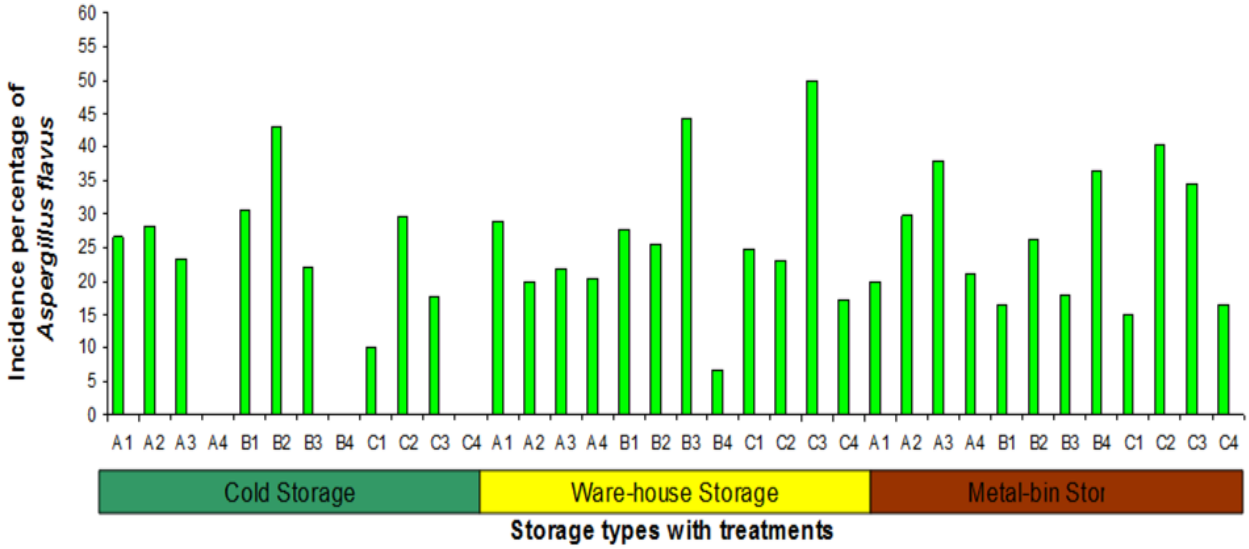


Fig. 2: Incidence percentage of *Aspergillus flavus* in maize seed under cold, ware-house and metal-bin storage conditions

A1 to A4: intact seed; B1 to B4: 5% damaged seed; C1 to C4: 10% damaged seed



also observed to be an influential factor. Maize with 10% and 5% damage was found with high aflatoxins, respectively. Overall, aflatoxin B_1 was detected in five, B_2 in four, G_1 in two and G_2 in one of the treatments.

The treatments, which exhibited production of aflatoxins, displayed prevalence of *A. flavus* with varied incidence, whereas *A. parasiticus* was absolutely absent. *A. flavus* was also isolated from the other treatments, where aflatoxins production was not recorded (Fig. 2).

Chulze (2010) concluded that some mycotoxins can contaminate maize before harvest and there level can be increase during storage, because agro-ecosystem is a complex and interrelated between biotic and abiotic factors in stored maize. Some previous studies in conformity to the present findings described fungal species *A. flavus* is responsible for production of aflatoxins (Manoch *et al*., 1988; Nepote *et al*., 1997; Medina-Martinez & Martinez, 2000). Four types of aflatoxins B1, B2, G1 and G2 were recorded in contrary to findings of Cotty and Cardwell, (1999) who reported for production of B1 and B2 in the presence of *A. flavus*. Although *A. parasiticus* was not observed but aflatoxin G1 and G2 were recorded, which has been reported as the general characteristic of *A. parasiticus* (Egel *et al.*, 1994), which proved the association of these aflatoxins with *A. flavus.* Sumbali (2001) found a high incidence of aflatoxins in stored maize seeds in India. Giorni *et al.* (2008) evaluated production of aflatoxins on maize during storage for up 21 days at 25ºC under a_w=0.95 and 0.92 up to 75% CO_2 resulted in an inhibition of the *A. flavus* population in grains. The efficacy of controlled atmosphere showed that treatment with 25% CO_2 reduced *A. flavus* development, but at least 50% CO_2 was necessary to reduce aflatoxins synthesis. Probst *et al.* (2010) determined aflatoxin content in maize from different provinces of Kenya and found 41% samples were below 20 ppb from Eastern Province, while a sample collected from Coastal Province exhibited 240 ppb of total aflatoxins, while aflatoxin levels reported by Firdous (2003) were up to 4.3 ppb in maize seeds from Pakistan. The high levels of present aflatoxins records may be attributed to wide storage types in the present investigations.

It was concluded that aflatoxins B1, B2, G1 and G2 has been observed to be a serious threat in maize in Pakistan. It is recommended to prefer cold and metal-bin storage, respectively along with physically intact maize to avoid the risk of aflatoxins.

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