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Anti-aflatoxigenic activity of *Punica granatum* and *Ziziphus jujuba* leaves against *Aspergillus parasiticus* inoculated poultry feed: Effect of storage conditions

Syeda Mona Hassan[a,*], Bushra Sultana[b], Munawar Iqbal[c,*], Saima Naz[d], Mazhar Abbas[e]

[a] *Department of Chemistry, Lahore Garrison University, Lahore, Pakistan*
[b] *Department of Chemistry, University of Agriculture Faisalabad, 38040, Pakistan*
[c] *Department of Chemistry, The University of Lahore, Lahore, Pakistan*
[d] *Institute of Chemical Sciences, Bahauddin Zakariya University, Multan, Pakistan*
[e] *Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan*

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ABSTRACT

The anti-aflatoxigenic activity of *Punica granatum* and *Ziziphus jujuba* leaves were investigated in *Aspergillus parasiticus* inoculated broiler feed. The commercial broiler feed was treated with plant leaves powder (5%, 10% and 15% w/w) and stored for the period of six months at different temperature and moisture conditions. Aflatoxins (AFTs-B1, B2, G1, G2) concentrations were determined at the end of each month by HPLC method and compared with control. *P. granatum* leaves inhibited AFTs production up to 100% up to four months of storage, whereas *Z. Jujuba* leaves exhibited AFTs production up to 3 months. The *P. granatum* and *Z. Jujuba* leaves AFTs inhibition in feed inoculated with *A. parasiticus* revealed that these plants are the potential source of antifungal activity, which could be employed to inhibit the stored feed degradation due to AFTs produced by *A. parasiticus*.

1. Introduction

The agriculture commodities, feed and food safety form AFTs contamination is a serious issue (Amirkhizi et al., 2015; Iqbal et al., 2015; Streit et al., 2012; Wu et al., 2015). Grains and protein are the main ingredients of the poultry feed. Molds growth is un-avoidable during crop harvesting, transportation, processing and storage due to moisture and resultantly, AFTs are produced in feed. AFTs not only deteriorate feed, but also adversely affect the health of birds, which utilize contaminated feed (Fallah et al., 2015; Fraga et al., 2007; Mariod and Idris, 2015; Torović, 2015; Wang et al., 2015). In South Asian countries due to increasing demand of protein, the poultry farming has gained importance (Mahesar et al., 2010). In Pakistan, more than 140 feed mills are operating with annual capacity of ~4 million tons of poultry feed (MLD, 2009). Since feed is used throughout the year and is stored for months for uninterruptable supply. Prolonged storage, temperatures variation, substrate composition, moisture and storage conditions play an important role in molds growth which contaminate the feed with AFTs (Fraga et al., 2007; Iqbal et al., 2015; Streit et al., 2012; Wu et al., 2015).

Aspergillus are the dominant species for the contamination of feed with AFTs and in view of their genotoxic, carcinogenic and hepatotoxic effects, the performance of living organism is badly affected (Iqbal et al., 2015; Stack and Carlson, 2003). Although, certain fungicides are known to inhibit the molds growth during storage, but fungicides might also cause toxicity and to ensure safety, there is a need to explore more effective, less expensive and eco-friendly strategies to control AFTs in feed (Iqbal et al., 2015; Streit et al., 2012). The biological approaches are regarded more effective and safer for AFTs control (Alaniz Zanon et al., 2013) and plants are the richest source of bioactive compounds (Adaramola and Onigbinde, 2017; Adaramola et al., 2016; Asif, 2015a,b,c,d,e,f, 2016; Hamid et al., 2016) and the antimicrobial activities of plants have been documented well elsewhere (El-Bendary et al., 2016; Gupta et al., 2012; Ramasubburayan et al., 2015; Saxena et al., 2013). In view of drawbacks of conventional treatments and current environmental pollution scenario (Babarinde et al., 2016; Babarinde and Onyiaocha, 2016; Gangadhara and Prasad, 2016; Iqbal and Khera, 2015; Jafarinejad, 2016; Jamal et al., 2015; Majolagbe et al., 2016; Peter and Chinedu, 2016; Qureshi et al., 2015; Rane et al., 2015; Sayed, 2015; Shindy, 2016; Ukpaka, 2016a,b,c,d), there is need to adopt the biological strategies to inhibit the fungal growth (Weaver et al., 2015). Therefore, the use of medicinal

* Corresponding authors.
E-mail addresses: s.monahassan@yahoo.com (S.M. Hassan), bosalvee@yahoo.com (M. Iqbal).

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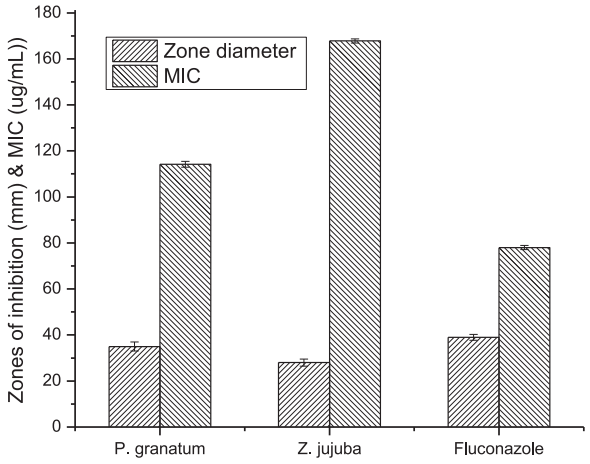

Fig. 1. Antifungal activity of medicinal plant leaves against *A. parasiticus* (MIC = minimal inhibitory concentration).

plants as an anti-aflatoxigenic will be of great interest since these are safer and eco-friendly in nature. The antifungal activities of *P. granatum* and *Z. Jujuba* are well known (Ferreira, 2007; Mahajan and Chopda, 2009), nevertheless, these are investigated for AFTs control, produced by *A. parasiticus* in stored poultry feed. Therefore, the motivation of present research work was to appraise the AFTs inhibitory effect of *P. granatum* and *Z. Jujuba* leaves in stored poultry feed.

2. Material and methods

2.1. Chemical and reagents

Culture media and standard discs were purchased from Oxoid Ltd., (Hampshire, UK). Immunoaffinity column (AflaTest® WB VICAM, USA), aflatoxin standards were obtained from Supelco (Bellefonte, PA, USA). Methanol, acetonitrile, *n*-hexane used were of analytical grade (Merck, Darmstadt, Germany). Trifluoroacetic acid (TFA) of Riedel-de Haen was used as derivitizing agent. Ultra-pure water with a resistivity of 18.2 MΩ cm from Milli-Q® system (Millipore) was used throughout the study for preparation of solutions.

2.2. Plant leaves collection

P. granatum and *Z. Jujuba* leaves were collected from Botanical Garden, Department of Botany, University of Agriculture, Faisalabad, Pakistan. The plant leaves were washed with water, dried under ambient conditions followed by oven drying at 70 °C to constant weight and grinded to fine powder. The powder was passed through 0.25 mm siever (OCT-DIGITAL 4527-OI) to obtained particles of uniform size.

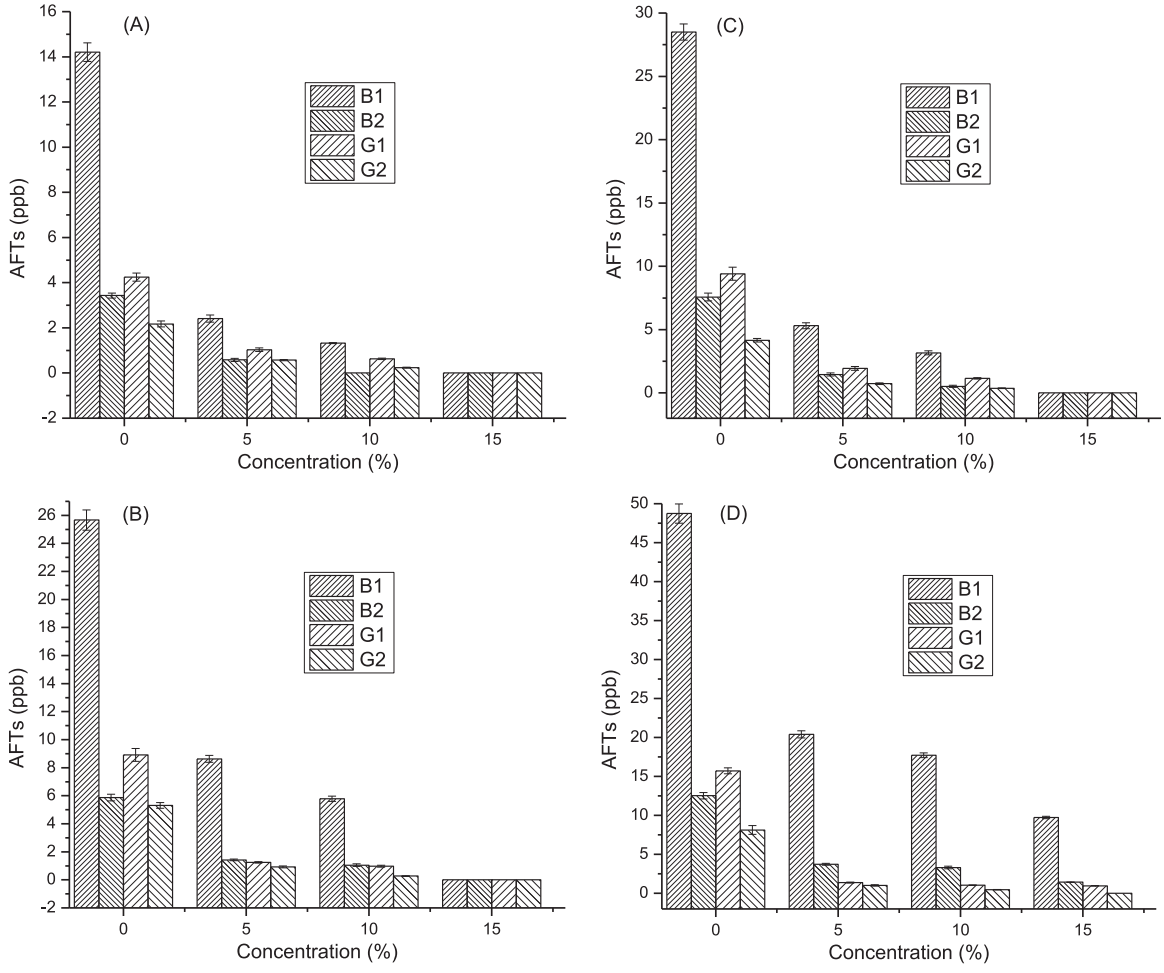

Fig. 2. (A) Aflatoxins (aflatoxin B1, aflatoxin B2, aflatoxin G1 and aflatoxin G2) concentrations (ppb) in feed treated with *Punica granatum* leaves powder at the end of 1st month of storage (stored at 16% moisture and 28 °C temperature levels) (B) Aflatoxins concentrations (ppb) in feed treated with *Punica granatum* leaves powder at the end of 1st month of storage (stored at 19% moisture and 31 °C temperature levels) (C) Aflatoxins concentrations (ppb) in feed treated with *Punica granatum* leaves powder at the end of 2nd month of storage (stored at 16% moisture and 28 °C temperature levels), (D) Aflatoxins concentrations (ppb) in feed treated with *Punica granatum* leaves powder at the end of 2nd month of storage (stored at 19% moisture and 31 °C temperature levels).