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SHORT COMMUNICATION/COURTE COMMUNICATION

Aflatoxin biosynthesis control produced by *Aspergillus flavus* in layer hens feed during storage period of six months

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Summary Aflatoxins (AFTs) are a group of closely related toxins that are produced by different fungus species. Food and feed contamination with AFT is a worldwide health-related problem. As a result of fungal attack, the food and feed resulted in a principal socioeconomic loss and toxins produced in feed and food items harm the humans and animals in different ways. The anti-aflatoxigenic effect *Psidium guajava*, *Ficus benghalensis*, *Gardenia radicans*, *Punica granatum* and *Ziziphus jujuba* leaves were evaluated against aflatoxins (AFTs), produced by *Aspergillus flavus* in layer feed during storage. Among the investigated medicinal plant leaves, *P. granatum* showed highly promising anti-aflatoxigenic activity and completely inhibited the AFTs (B1 and B2) production over storage period without compromising the nutritive quality of feed (ash, protein, fat, fiber, Fe, Ca, P and K contents). Leaves of *F. benghalensis* and *Z. jujuba* were also effective however, higher concentration (15%) inhibited the AFTs production up to 99% and also maintained nutritive quality of feed. *G. radicans* was found least effective in controlling the AFTs production. Results revealed that all plant leaves were effective in controlling AFTs production in layer feed over the storage period of six months and these plants are potential candidate to replace the fungicides used to protect feed and other agricultural commodities from AFTs production during storage.

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Introduction

The poultry farming has gained importance throughout the world since protein demand is increasing day by day [1]. In Pakistan, poultry farming has also been established and is supporting the economy of the country in parallel to other industries. At present, approximately 140 mills are operating for poultry feed preparation and four million tons of poultry feed is produced annually in these feed mills to nourish poultry birds. It is estimated that poultry forming contributing 4.81% in Pakistan economy that is 9.84% for total livestock [2]. Poultry feed comprising peanut, cottonseed, cereal grains and proteins like soya been, corn gluten, copra and sunflower [3,4]. Since the feed is used throughout the year and is stored for months. Factors, such as prolonged drought, temperatures variation, substrate composition, storage time and storage conditions are the major sources of fungi contamination, which produce AFTs in stored feed [5,6]. According to recent European Union survey [7] and AFTs contamination [6] of grains, crops, dairy products, it has been found that most of the collected sample were contaminated with AFTs and for the preparation of poultry feed grains are used and the production and contamination of poultry feed is also associated with AFTs. The mouls and AFTS not only degrade the feed quality, but are also associated with health issue of poultry birds and human being [3].

A. flavus is one of the dominant microbes, responsible for feed and other agricultural commodities contamination. Among mycotoxins, AFTs have received greater attention due to their carcinogenic, genotoxic, teratogenic, dermato-, nephro- and hepatotoxic effects [5,6]. Many countries, especially in tropical region are facing difficulties to control AFTs contamination and in spite of precautionary measures, absolute safety has never been achieved. Although, certain fungicides have been known to inhibit the *A. flavus* growth during storage, but consumer apprehension about possible risks linked with fungicides resulted in extensive search for more effective and safer control strategies. The chemical detoxification involves the use of chemical agents [8], whereas physical methods comprises radiation, microwave heating that have been used for the detoxification of AFTs [9] On the other hand, biological approaches are more effective and safer for AFTs and in this regard, plants are the rich sources phytochemicals and their fungicidal activities have been documented well [10]. Therefore, the use of medicinal plants as anti-aflatoxigenic will be interesting since these are safer versus chemical or physical treatments [8,9].

In spite of fungicidal properties of medicinal plants, nevertheless, these are investigated for AFTs control in broiler stored feed. Therefore, the motivation of present research work was to appraise the AFTs reduction, produced by *A. flavus* during storage period. The medicinal plant i.e., *P. guajava*, *F. benghalensis*, *G. radicans*, *P. granatum* and *Z. jujuba* were selected and appraised there anti-aflatoxigenic activities. The treatment efficiency was evaluated on the basis of AFTs reduction and effect on nutritional value of stored broiler feed.

Material and methods

Chemical and reagents

The media and standard were purchased from Hampshire, UK. Immunoaffinity column (AflaTest® WB VICAM, USA). Bellefonte, USA provided AFTs standards. All other reagents and chemicals i.e., methanol, trifluoroacetic acid, acetonitrile, *n*-hexane purchased from Merck, Darmstadt, Germany.

Plant collection and extraction

The *P. guajava*, *F. benghalensis*, *G. radicans*, *Punica granatum* and *Z. jujuba* plants were taken from University of Agriculture, Botanical Garden and identified from Department of Botany, UAF. The selected plant leaves were washed, dried under ambient conditions any oven drying at 70 °C to constant weight and then, grinded to fine powder.

Preparation of antifungal extracts

Leaves antifungal extracts were prepared by dissolving 10 g powder in 100 mL methanol (80%) and shaken for 24 h at room temperature and then, filtered to separate the extract from residues. The solvent was evaporated under vacuum (EYELA, N-N Series, Rikakikai Co. Ltd. Tokyo, Japan) and stored in refrigerator.

Preparation of inoculum

The fungal strains were procured from CMS, UAF and identified from Institute of Microbiology, UAF. The microbes were cultured on potato dextrose agar slant and for sporulation, microbe was incubated for 7 days at 28 °C temperature. Then spores were collected 0.1% Tween 80 solution in water. At the time of harvesting, the spores count was 1×10^7 spores/mL (counted in nebauer chamber with microscopy), which was preserved at -4 °C.

Disc diffusion assay

For antifungal activity, 20 mL PDA solution containing 1×10^7 spores/mL was spread on sterilized Petri plate and 6 mm sterilized discs were loaded with extract (50 μL) and incubated at 28 °C for 48 h [11]. Fluconazole 30 μg/disc was used as positive control. The zone of inhibition diameter was measured (mm) by zone reader.

Minimum inhibitory concentration (MIC) determination

For MIC estimation, 100 μL of extract (10 mg/mL) was transferred to 96 well plates and 50 μL of sabouraud dextrose broth (SDB) was added to the wells. A series of dilutions were performed in descending concentration order. Finally, 10 μL of fungal suspension (1×10^7 spores/mL) was added to each well. The plates were incubated at 28 °C for 48 h.

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