SHORT COMMUNICATION

Mycotoxins in *Zea mays*, their quantification and HPLC analysis of physico-biological detoxification

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ABSTRACT

Present research delves in the isolation, extraction and identification of mycotoxins from ten corn samples collected from the northern province of Pakistan. Average concentration of aflatoxin B1 and B2 by HP-TLC found in all corn samples was 27.87 and 1.35 \(\mu\)g/kg, respectively. Following HP-TLC, detoxification of the identified and isolated mycotoxin was performed, which was analyzed by HPLC. Screening of mycoflora exhibited *Aspergillus niger* and *Fusarium* as the most dominant fungal strains. Aflatoxin B1 was physically detoxified under UV-Lamp and direct sunlight displaying detoxification percentage of 48% and 99%, respectively. Biological detoxification involved the use of botanicals such as neem leaves, garlic and ginger powder, which portrayed an approximate detoxification of 70% from corn samples. Current research concludes that the tested physical and biological methods can be easily adopted at field and storage rooms after the harvesting of crops to avoid fungal contamination and subsequent food spoilage.

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1. Introduction
Corn (Zea mays L) is a main cereal crop cultivated under an area of more than 118 million hectares. The main fungal species, which infect the corn crop, are Aspergillus flavus, Aspergillus niger and Fusarium (Moore et al. 2017). These fungi species grow well in the range of 19–35 °C and produce maximum Aflatoxins at 28 °C (Wang et al. 2019). Various varieties of corn are cultivated in Pakistan but most important are corn hybrid, which generate a yield of 12 ton per hectare as compared with other corn varieties that have less production (Jan et al. 2018). In Pakistan, each year food production is affected due to microbial contamination (Rafique et al. 2018). Aflatoxin B1 is the most potent natural carcinogenic compound known and is usually the major aflatoxin produced by toxigenic strains (Carter et al. 2019). A wide range of commodities can be contaminated with mycotoxins both preharvest and postharvest (Sserumaga et al. 2020).

Present research focuses on the analysis, determination and detoxification of mycotoxin, aflatoxin B1 from corn samples. Ten corn samples, collected from the local shops were used for the extraction and quantification of aflatoxins. Furthermore, mycotoxin decontamination was performed by physical and biological means including UV treatment, direct solar radiation and treatment with neem leaves, ginger and garlic powder. It was hypothesized that these methods may prove to be highly efficient and cost effective in the detoxification of corn samples. The research was designed to single out a technique, among many, which is efficient and effective for the detoxification of mycotoxins whilst being cost-effective and environment friendly.

2. Results and discussion
2.1. Isolation and identification of fungi in corn samples
Fungal identification was done based on the nature and color of fungal colonies. Visual identification revealed that A. niger displayed black, thick colony while Fusarium displayed rough, green colonies (Supporting Information Figure S2) (Benoit-Gelber et al. 2017; Hussein et al. 2017). Results of isolation and identification of fungi associated with corn revealed Aspergillus species including A. niger and A. flavus. Fusarium sp. was also isolated, however, A. niger was the most dominant fungi associated with corn grains due to its high occurrence percentage (84%). Occurrence of A. flavus reached only 5%. While Fusarium sp. was found 11% in corn grains (Supporting Information Figure S2).

2.2. Detection and quantification of aflatoxins by HP-TLC
All of the samples of corn were fractionated on high performance thin layer chromatography (HP-TLC; Supporting Information Table S1). Each sample was compared with aflatoxin standard. Retention factor, size of spot and intensity of brightness of spot of samples were observed. During comparison, eight samples possessed aflatoxin B1 (AFB1) concentration greater than the permissible value, i.e. 20 ppb while one sample contained 8.104 ppb toxin concentration whereas one sample did not show any
aflatoxin presence. Aflatoxin B2 was detected in only half of the samples. Highest level of aflatoxin B1 was found in corn samples 5 and 8 to be 48.48 µg/kg while the lowest level was detected in sample 4 to be 9.09 µg/kg. AFB1 contamination in corn samples was found to be high than aflatoxin B2. Average level of AFB1 contamination was 27.87 µg/kg while that of AFB2 was 1.35 µg/kg. Highest concentration of AFB2 was detected in three samples of corn, i.e. 1, 2 and 7 to be, 3.75 µg/kg. Lowest level of AFB2 was detected in corn sample 9, i.e. 0.05 µg/kg. Aflatoxin G1 and G2 were not detected in any of the samples. A study on mycotoxin contamination on maize hybrids, which were infected with *Fusarium proliferatum* exhibited the use of HPTLC to confirm the quantitative data (Pascale et al. 1999). Truckssess et al. (1990) also analyzed aflatoxins in corn by HPTLC. HPTLC is a popular technique due to convenience and reduced operating costs.

2.3. HPLC analysis of UV radiation treatment

The result of detoxification through UV treatment exhibited reduction in height, area and retention time, which can display the efficiency of this method (Supporting Information Figure S3). HPLC chromatogram of UV treatment for 20 min on corn for detoxification displayed Aflatoxin at retention time 1.868 min of peak height 14,790 at an area of 190,365. The corn sample was further detoxified and analyzed after 40 min. It was observed that chromatogram displayed Aflatoxins at retention time 1.903 min with a peak height of 8482 at an area of 117,874. After further detoxification for 60 min, it was analyzed that Aflatoxin were detected at retention time 2.085 min with peak height of 5118 at an area of 97,847. When aflatoxins present in corn samples absorb UV radiation, they get activated resulting in increase in their vulnerability to degrade. UV treatment of Aflatoxin B1 resulted in degradation products with molecular weights, 316 and 302 g/mol. Percentage decontamination of aflatoxin from corn sample after 40 min was 38% while after 60 min, it elevated to 48.6%. Results portrayed the escalation in degradation efficiency with the increase in time duration.

Culturing of corn sample exhibited that 60% of corn was detoxified by UV lamp after 20, 40 and 60 min indicating that UV treatment is useful and impressive for detoxification of corn from mycotoxins. The HPLC chromatograms (Supporting Information Figure S3) display small peaks other than the main aflatoxin peak indicating the formation of daughter products.

2.4. Solar treatment

HPLC chromatogram of solar treatment for 2 days on corn sample for detoxification displayed Aflatoxins at retention time 1.887 min with peak height 9759 at an area of 135,508. Corn sample was further detoxified and analyzed after 4 days. It was observed in the HPLC chromatogram that Aflatoxins were detected at retention time 1.875 min with peak height 558 at an area of 1920. The corn sample was further detoxified by direct sunlight and analyzed after 6 days, which displayed Aflatoxins at retention time 1.908 min of peak height 60 at an area of 1237 as shown in Supporting Information Figure S4. The result of detoxification through solar treatment exhibited the reduction in height, area and retention time, it can be concluded that this method is effective.
and impressive because farmers can use it easily to remove toxins from their corn fields. Direct solar treatment exhibited a rapid detoxification in corn samples. 98.5% aflatoxins were detoxified after 4 days while 99% decontamination was observed after 6 days. HPLC chromatograms clearly exhibit the formation and increase in the smaller peaks showing the degradation products at all days (Supporting Information Figure S4).

Following exposure to sunlight for 2, 4 and 6 days the corn sample was cultured to assess the decontamination by direct solar treatment.

2.5. HPLC analysis of biological detoxification

Biological detoxification of corn samples was performed by utilizing botanicals including garlic powder, ginger powder and neem leaves for 1 week.

Supporting Information Figure S5 (A) exhibits the retention time of corn sample by garlic treatment at 1.975 min with peak height, 12,840 and area, 235,259. Supporting Information Figure S5 (B) represents chromatogram for the mycotoxin decontamination by ginger treatment at retention time of 1.980 min, peak height, 15,148 and area, 265,959. Supporting Information Figure S5 (C) displays the chromatogram of corn mycotoxin detoxification by neem leaves at retention time 2.175 min, peak height, 11,459 and area, 175,876. It was observed that the use of such organic and natural botanicals for mycotoxin detoxification results in environmental friendly and safe remedial strategies without the utilization of harmful chemicals. Such means of decontamination no negative impact on crop and consequently on human and animal health. The corn sample was cultured following treatment with botanicals for 1 week. Culturing of corn after detoxification exhibited that there is 70% detoxification of fungi by garlic treatment, 60% by ginger treatment and 80% by neem treatment. All the botanicals displayed significant detoxification percentages exhibiting their effectiveness for such techniques.

3. Experimental

The assessment of extracted mycoflora was performed through HP-TLC. Detoxification of the contaminated samples was done by physical and biological means and examined through HPLC (Comprehensive research methodology provided as supplementary document).

4. Conclusion

The current study deliberates mycotoxin detection and analysis techniques, which can be used as simple and rapid screening tests for cost-effective control of food diseases. Present investigation has demonstrated exorbitantly easy, cost effective and environmental friendly detoxification strategies for aflatoxin decontamination from corn samples. Sunlight degraded aflatoxins in corn samples, consequently, indicating it to be an effective method for detoxification of aflatoxins. Since, sunlight is entirely natural, hence, it is expected that the corn samples will retain their nutritional quality even after treatment. Sun-drying is helpful because long-wave UV radiation from the sun
detoxify the toxin up to 90% on the surface of grains. This is a great advantage for the use of physical methods as detoxification means. Detoxification under direct sunlight proved to be the easiest technique to follow for researchers as well as farmers, equally. It is suggested that the food industries should also prefer to opt for such highly efficient and cost-effective techniques, since these methods are also economically stable whilst being environmentally benign. This research thus encourages the use of sunlight, among other physical methods, as an efficacious technique to detoxify aflatoxins.

Disclosure statement
No potential conflict of interest was reported by the authors.

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