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UTILIZATION OF FRUIT PEELS TO INHIBIT AFLATOXINS SYNTHESIS BY *ASPERGILLUS* SPECIES: A BIOTREATMENT OF TWO PULSES *CICER ARIETINUM* AND *VIGNA RADIATA* FOR SAFE LONG–TERM STORAGE

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Abstract

Aflatoxins are assembly of nutrition poisons which are lethal, cancer–causing metabolites mostly transmitted by specific strains of micromycetes. Aflatoxins are produced during storage of grains. Reduction of aflatoxins productions in chick pea (*Cicer arietinum*) and mung bean (*Vigna radiata*) was analyzed during stockpiling of selected cereals utilizing peels of *Citrus sinensis* and *C. limon*. For this reason, *C. arietinum* and *V. radiata* were inoculated with fungal spores and balanced out by lemon and orange peels powder by using various trials. Test samples were stored at 30°C for six months. Aflatoxins were analyzed by high performance liquid chromatography (HPLC) which demonstrates that these natural products are intense preventer of aflatoxins production in pulses and show decrease of aflatoxins. Lemon peels showed maximum inhibition of 20% in chick pea than mung bean whereas orange peels showed more inhibition of 28% in mung bean than chickpea.

Key words: Biocontrol; MEA; Micromycetes; Mold.

Introducation

Rural items including corn, fiber, peanuts, and ground nuts etc. tainted by aflatoxins produced by pathogenic fungi had increased general significance because of their pernicious consequences for human and creature wellbeing just as their significance to worldwide exchange (Severns *et al*., 2003). Micromycetes (fungi) attack oats and grains during storage and can cause two problems viz., grain deterioration from contagious development or molds and the generation of harmful mycotoxins (Ferreira *et al*., 2013; Havrlentová *et al.*, 2021). Among micromycetes there are three leading genera *Aspergillus*, *Fusarium* and *Penecillium* that are measured to create fungal poisons from crop development to storing (Al-Hazmi *et al*., 2010) while *Aspergillus* is in charge of aflatoxin assembly (Amaike & Keller, 2011; Pfliegler *et al*., 2020).

There are various types of aflatoxins (AFs) however AFB1, AFB2, AFG1 and AFG2 present normally in many sorts of nourishments in tropological nations. Aflatoxin AFB1 is the most dominant cancer–causing agent (Romani, 2004; Kumar *et al.*, 2021) and a global intervention for investigation on cancer has grouped such aflatoxins in class 1A cancer–causing agent for people (Marchese, 2018). Various Factors like dampness content, activity of water, tainting level and toxicoid capability of organism, temperature, stockpiling term and nature of substrate impact the generation of mycotoxins (Nafeesa & Salma, 2006).

Although, certain fungicides are known to repress the molds development during storage, yet fungicides may cause lethality and to guarantee wellbeing increasingly viable, there is a need to investigate progressively viable, more affordable and eco–accommodating techniques to control AFTs in feed (Iqbal *et al*., 2015). Natural methodologies are respected progressively effective and more secure for AFTs control and plants are the most excessive sources of bioactive mixes (Zanon *et al*., 2013). Orange (*Citrus sinensis* (L.), Family: Rutaceae) and Lemon (*Citrus limon* (L.), Family: Rutaceae) peels have significant result in this scenario due to presence of flavedo, albedo, cellulose, hemicellulose, lignin, gelatin, chlorophyll pigments, and other low–atomic weight mixes (for example limonene), as well as volatile and nonvolatile portions of basic oils and seasoning mixes and furthermore for improving the timeframe and wellbeing of different food items (Khaskheli *et al*., 2011; Garcia–Perez *et al*., 2008).

Cicer arietinum (Chickpea) and *Vigna radiata* (mung bean) are the best nutritive among the eatable pulses and widely utilized as protein added substance to starchy food (Sastri, 1962) but their fungal diseases are the serious issue in sub–continent (Dawar *et al*., 2007). Therefore, aim of present investigation is to inhibit the aflatoxins production by using fruit peels in *C. arietinum* and *V. radiata* for long term storage and to compare the inhibitory potential of orange and lemon peels against aflatoxin production.

Materials and Methods

Isolation of fungi and inoculum preparation: Pulses of selected cereals were placed on Malt Extract Agar (MEA) medium for isolation of fungi that contaminate these cereals. Petri plates were sealed in order to avoid contamination and labeled and placed at room temperature for 7 days in laminar flow. Then fungal cultures were examined and identified after macroscopic and microscopic characterization of colonies. Isolated

species were identified after recording parameters like shape of spores, hyphae colony size and colony morphology and consulting available published literature. Spore inoculum was prepared one day before its use and preserved at 4°C.

Inhibitory potential of fruit peels on aflatoxins production in selected pulses

Choice of fruit peels for bio treatment: *Citrus limon* and *C. sinensis* were chosen for bio treatment. Fruits were collected from local markets. Peels of these natural products isolated, dried and ground to fine powder by utilizing a research facility processor and put away in polythene bags at 4°C for later use.

Moisture content and pretreatment of selected pulses: About 2 kg of each kind of cereal grain samples *Cicer arietinum* (chickpea) and *Vigna radiata* (mung bean) were taken from a local market. Their moisture contents were estimated by following Reeb and Milota (1999) after drying replicates of 5 g of grains. Pulses were separately washed with water and dried in an oven at 38–65°C. Each portion of pulses was divided into 10 equal parts, each containing 200 grams of grains of single variety. Each part was autoclaved independently to kill microbes and packed in air tight plastic bags aseptically under laminar air flow chamber. Pulses were moistened with sterilized distilled water to increase the required humidity level to 21% and inoculated by mingling 4 ml of spore suspension of selected fungus to each pack independently.

Maintenance of cereals with treatment of peels: Orange and lemon peels in ground configurations at three proportions viz., 5%, 10%, 20%, w/w were added independently to every plastic bag containing 200 g of mixed pulses and shaken completely to merge plants powder consistently with grains. Specimens deprived of treatment (without peels) were utilized as control. The treated and control samples with moisture level of 21% were stored at 28°C for a time of a half year.

Extraction and analysis of aflatoxins: Extraction of aflatoxins from pulses was done by following Beltrán *et al.,* (2011) with certain adjustments. A powdered test (20 g) of cereal grains was taken in a 250 mL conical flask and concentrated utilizing arrangement (80 mL) of acetonitrile–water (84:16) in a shaker at 37°C for 90 mins. The concentrate was separated through Whatman filter paper and filtrate was focused under less pressure at 50°C to conclusive size of 2–5 ml. The subjective investigation of concentrates for the proximity of aflatoxins were done by Thin layer chromatography (TLC) strategy while measurement of aflatoxins was done by High performance liquid chromatography (HPLC) system.

Quantitative determination of AFTs: The quantity of aflatoxins was found by HPLC, extract sample was diluted with 20 mL of deionized water and sample was passed through aflatest immune affinity column at a flow rate of 2 mL/min. HPLC system used was by Shimadzu LC–10 A series (Shimadzu, Japan), equipped with Discovery HS C–18 Column of 250 mm–4.6 mm, column oven (CTO–20 A Shimadzu Japan), system controller unit (SCL–10 A), UV–Vis detector at 180–550 nm. Methanol acetonitrile and distilled water at the proportion of 23:23:54 were used as mobile phase with a flow rate of 1.5 mL/min. Column temperature was adjusted at 35°C.

Statistical analysis

All determinations were made and the results of numerous parameters were described. The results of the peels treatment were shown in the form of tables and graphs. The results were analyzed statistically by using Microsoft Office Excel 2010.

Results

Fungal isolates: Among isolated fungal colonies, dominant micromycetes reported in chickpea were *Aspergillus flavus* Link, *Aspergillus fumigatus* Rai. and *Aspergillus niger* Tiegh. while dominant micromycetes reported in mung bean were *Aspergillus fumigatus* Rai., *Aspergillus* sp. *Aspergillus paraciticus* Speare. and *Fusarium* sp.

Competency of fungi for production of aflatoxins in cereals: The aflatoxigenic strain of *Aspergillus* species were utilized because of the way that *Aspergillus* is the most undesirable species worldwide and can assault under ideal states of temperature and moisture on different types of pulses. Proximity of aflatoxins in samples (with no supplement) put away at 21% moisture and 30°C temperature demonstrated the nearness of B and G kinds of aflatoxins and by contrasting the Rf estimations of tested samples with the measures four sort of aflatoxins for example AFB1, AFB2, AFG1 and AFG2 were distinguished.

Quantitative determination by HPLC demonstrated that the complete aflatoxins substance collected by *Aspergillus* species in control tests of grains were 7.626 ng/g in chickpea, 311.12 ng/g in mung bean. Results demonstrated that chickpea is progressively rich to aflatoxins generation followed by mung bean. Among absolute aflatoxins substance gathered in pulses; aflatoxin G1 was at largest amount of 19.5, 20.3 and 10.4 ng/g, separately, and aflatoxin B1 was 8.12 and 5.2 ng/g, individually; while aflatoxin G2 was identified in limited quantity. Measurable examination demonstrated that the measure of aflatoxin G1 delivered by tried strain in two unique cereals was observed to be fundamentally. The sprouting and development of shape spores and mycelium were influenced by the sort of dull nourishment and the accessibility of supplements in type of protein and carbohydrates.

Filtrate of fungal isolate showing Aflatoxins: Filtrate of *Aspergillus* species is shown in (Fig. 1a & 1b). More samples contained AFGI while only one sample contained AFB1.

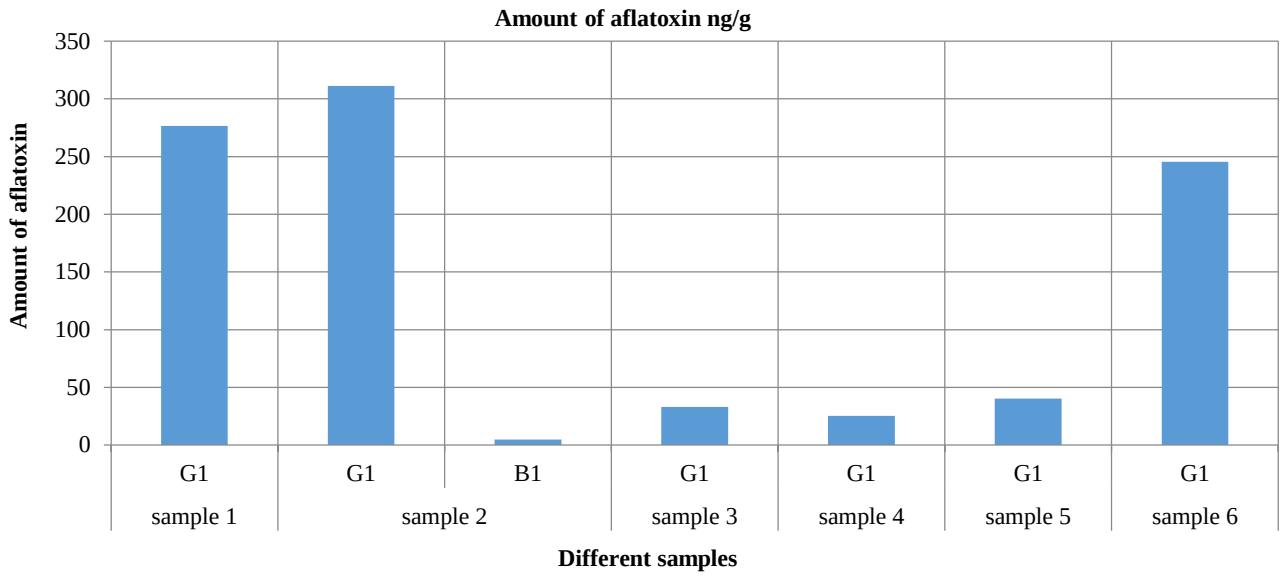

Fig. 1a. Aflatoxins in different samples.

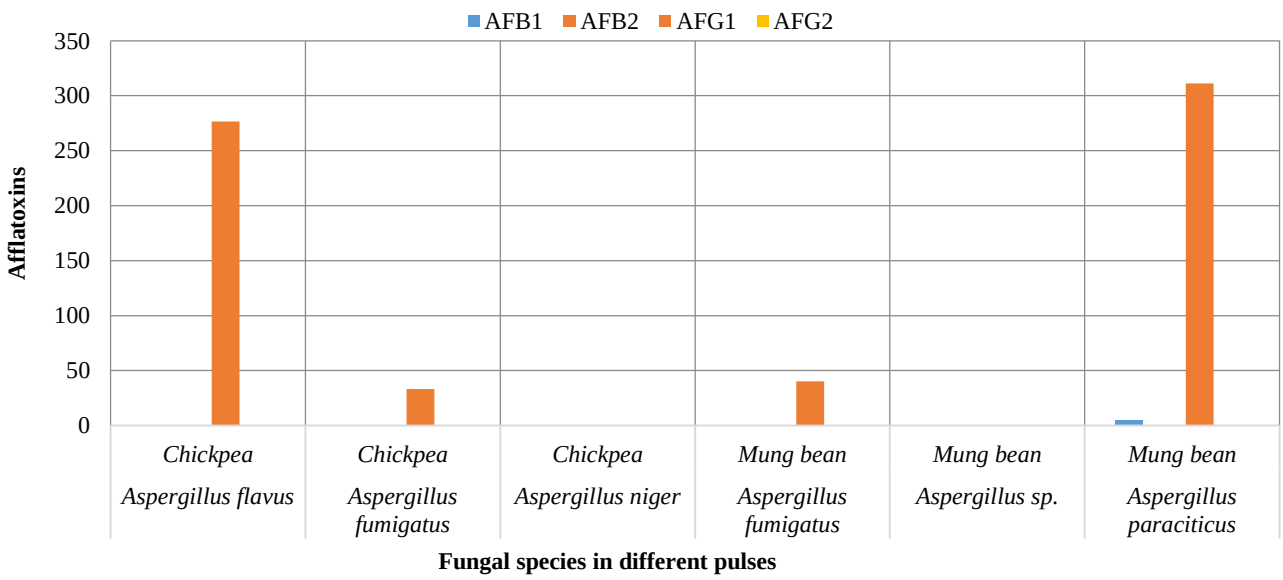

Fig. 1b. Aflatoxin concentration in pulses containing different micromycetes.

Efficiency of fruit peels in reduction of aflatoxins in chickpea: The control sample of chickpea containing aflatoxin G1 amount is 276.62 ng/g, 230.4 ng/g, 256.5 ng/g, 180.4 ng/g, 148.2 ng/g and 172.1 ng/g in first, second, third, fourth, fifth and sixth month respectively. Reduction shown in chickpea aflatoxins G1 with the inoculation of orange peels is 184.2 ng/g, 156.3 ng/g, 125.4 ng/g, 102.1 ng/g, 76.12 ng/g and 33.14 ng/g in first, second, third, fourth, fifth and sixth month respectively (Fig. 2a). Similarly, reduction shown in chickpea aflatoxins G1 with the inoculation of lemon peels was 45.42 ng/g, 38.1 ng/g, 34.7 ng/g, 28.3 ng/g, 21.1 ng/g and 20.2 ng/g in first, second, third, fourth, fifth and sixth month respectively (Fig. 2b).

Reductions in aflatoxin G1 in chickpea stabilized by orange peels were 66%, 67%, 48%, 56%, 51% and 20% in first, second, third, fourth, fifth and sixth month respectively while reductions in aflatoxin G1 in chickpea stabilized by lemon peels were 16%, 16%, 13%, 15%, 14% and 11% in first, second, third, fourth, fifth and sixth month respectively (Fig. 4). It showed that the lemon peels are more effective than orange peels in reducing aflatoxin G1 in chickpea.

Efficiency of fruit peels in reduction of aflatoxins in mung bean: The control sample of mung bean containing aflatoxin G1 amount was 311.12 ng/g, 287.4 ng/g, 237.3 ng/g, 184.5 ng/g, 156.2 ng/g and 140.7 ng/g in first, second, third, fourth, fifth and sixth month respectively. Reduction shown in mung bean aflatoxin G1 with the inoculation of orange peels was 158.1 ng/g, 137.2 ng/g, 120.5 ng/g, 101.4 ng/g, 70.3 ng/g and 40.29 ng/g in first, second, third, fourth, fifth and sixth month respectively (Fig. 3a). Similarly, reduction shown in mung bean aflatoxin G1 with the inoculation of lemon peels was 245.42 ng/g, 220.1 ng/g, 189.4 ng/g, 156.5 ng/g, 120.4 ng/g and 102.3 ng/g in first, second, third, fourth, fifth and sixth month respectively (Fig. 3b).

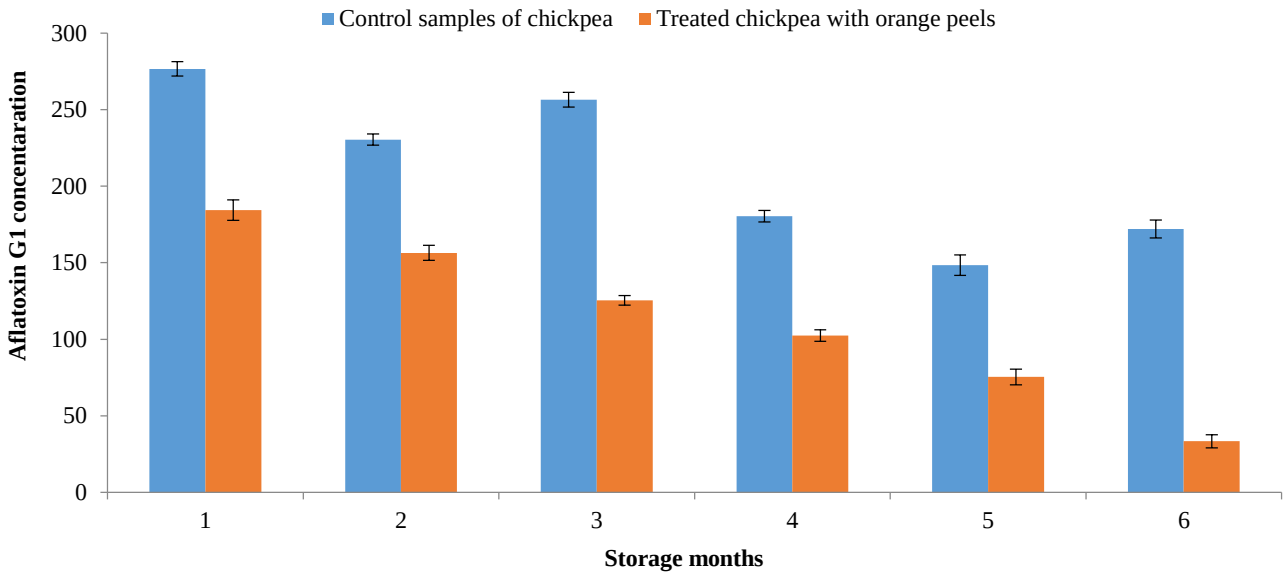

Fig. 2a. Aflatoxins in chick pea stabilized by orange peels during 6 months of storage.

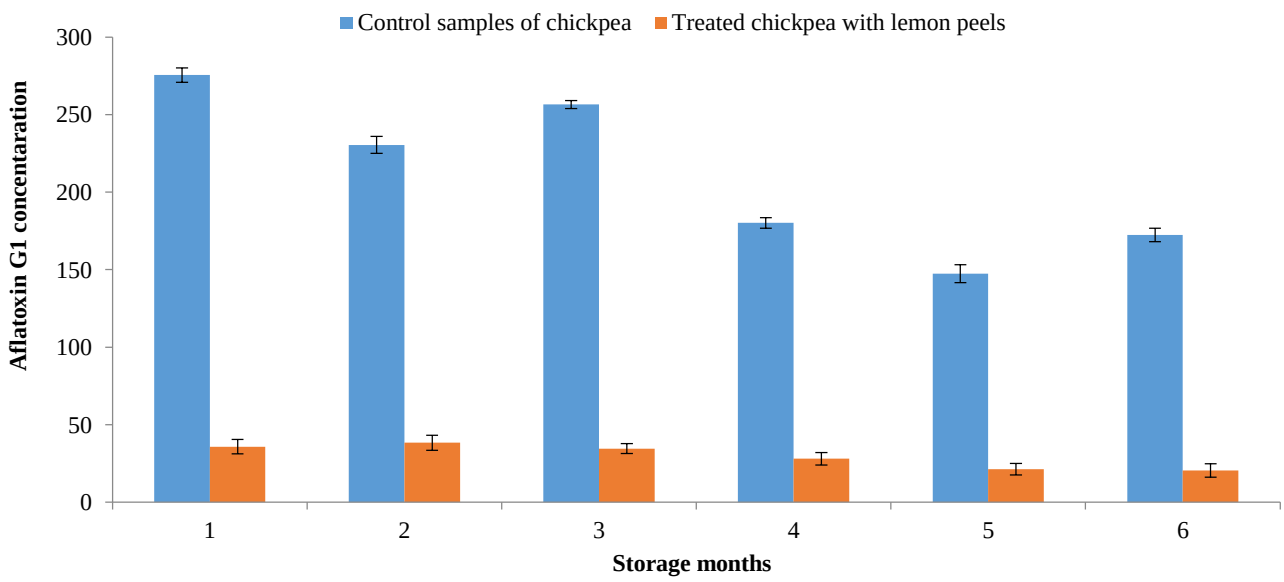

Fig. 2b. Amount of aflatoxins in chick pea stabilized by lemon peels during 6 months of storage.

Reductions in aflatoxin G1 in mung bean stabilized by orange peels were 50%, 47%, 43%, 54%, 44% and 28% in first, second, third, fourth, fifth and sixth month respectively while reductions in aflatoxin G1 in mung bean stabilized by lemon peels were 78%, 76%, 79%, 84%, 76% and 72% in first, second, third, fourth, fifth and sixth month respectively (Fig. 5). It showed that the orange peels are more effective than lemon peels in reducing aflatoxin G1 in mung bean and lemon peels show more inhibition in chickpea aflatoxins.

Discussion

Orange and lemon peels were connected to two cereals to explore their inhibitory impact on aflatoxin (B1, B2, G1 and G2) generation by *Aspergillus* species. The inhibitory impact of leaves was assessed by looking at aflatoxins substance in control samples (with no peels) with that created in peels treated samples. The generation of aflatoxins in treated examples was observed to be less when contrasted with control tests during a half year of storage. Sultana, (2015) stated that the growth of *Aspergillus parasiticus* and aflatoxins production were inhibited during storage of three important cereals (wheat, maize and rice) using leaves of neem (*Azadirachta indica*) and kikar (*Acacia nilotica*). Neem leaves fully inhibited all types of aflatoxins synthesis for 4 months in wheat and for 2 months in maize while in rice inhibited synthesis of only B2, G1 and G2 aflatoxin for 3 months. Kikar leaves fully inhibited aflatoxin B2, G1 and G2 for 3 months in wheat, and for 2 months in maize. Among two investigated plants, neem leaves were found more effective for preventing the production of all types of aflatoxins in cereals' long–term storage.

During this investigation, both selected plant treatments restrained the union of aflatoxins in grains. As a rule, an immediate relationship was seen between

the connected plant focus and their inhibitory impact on aflatoxins production. Our findings that *Aspergillus* species was responsible for the production of four types of aflatoxins i.e. BI, B2, G1 and G2 are in fair agreement with the investigations of (Sharma and Sharma, 2012) who have reported the production of aflatoxin BI, B2, G1 and G2 by *A. parasiticus*.

Result of present research showed that aflatoxin G1 is present in both chickpea and mung bean while aflatoxin B1 is only present in mung bean. Aflatoxin B2 and G2 were absent in all samples of mung bean and chickpea. Zahra *et al.* (2012) studied aflatoxins in red chilli samples in which 48 samples were positive for aflatoxin B1 out of 80 samples. Aflatoxin B2 was detected only in three samples. Aflatoxin G1 and G2 were absent in all samples.

Results of present research work demonstrate that lemon and orange peels inhibit the formation of aflatoxins in a half year in both chickpea and orange peels. Lemon peels showed more aflatoxin inhibition of 20% in chick pea. Orange peels showed more inhibition of 28% in mung bean. These peels can be presented in agroindustry advertise as a characteristic, protected and less expensive added substance to restrain aflatoxins generation during the storage of pulses. Naseer *et al.* (2014) and Ismail *et al.* (2021) stated that expansion of pomegranate–peels controls the aflatoxins generation to 100% during many month–stockpiling of rice whereas lemon–peels additionally indicated inhibition for ninety days. Studies demonstrated that both organic product squanders are predominant preventer of aflatoxins formation in rice, valuable for a more secure and longer stockpiling of rice.

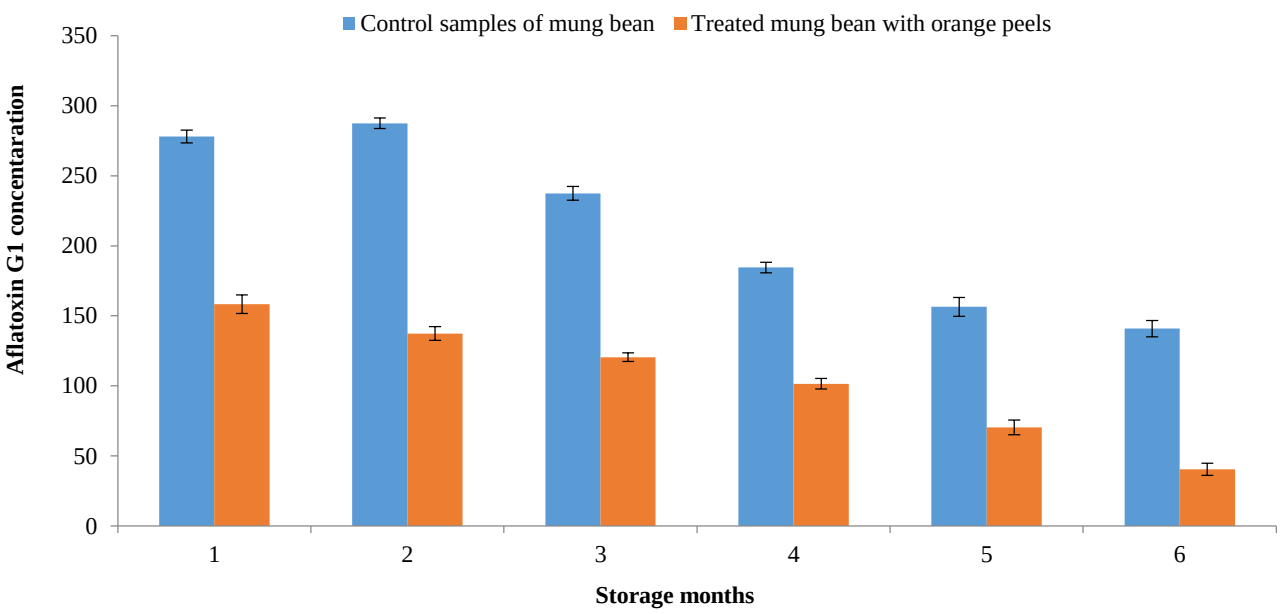

Fig. 3a. Amount of aflatoxins in mung bean stabilized by orange peels during 6 months of storage.

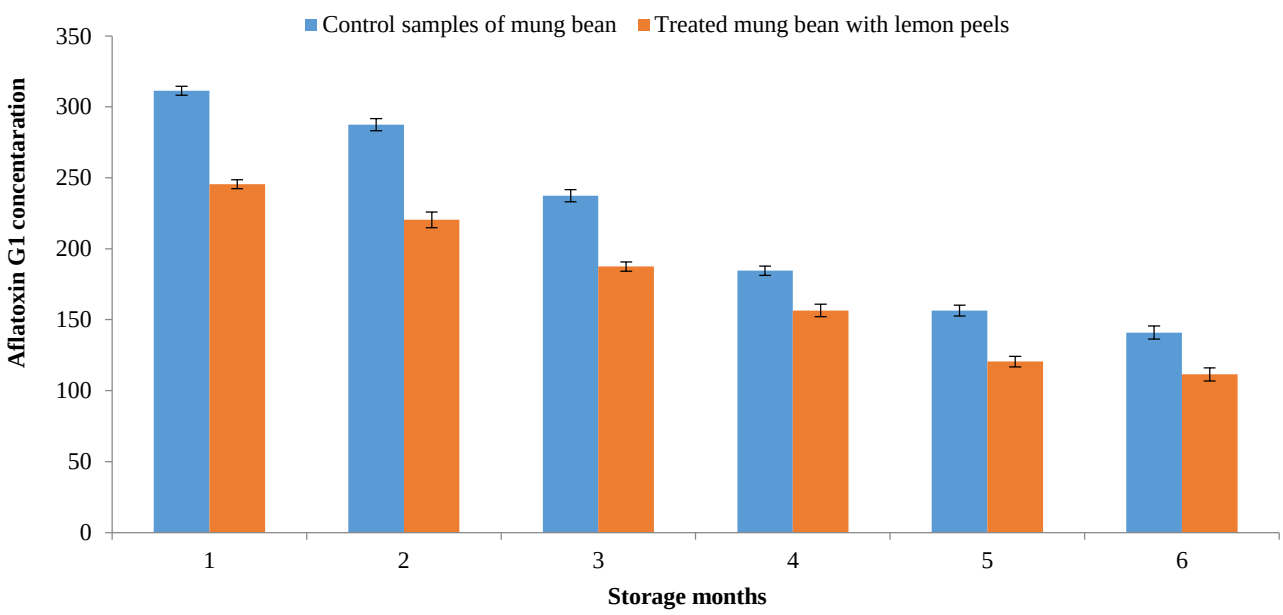

Fig. 3b. Amount of aflatoxins in mung bean stabilized by lemon peels during 6 months of storage.

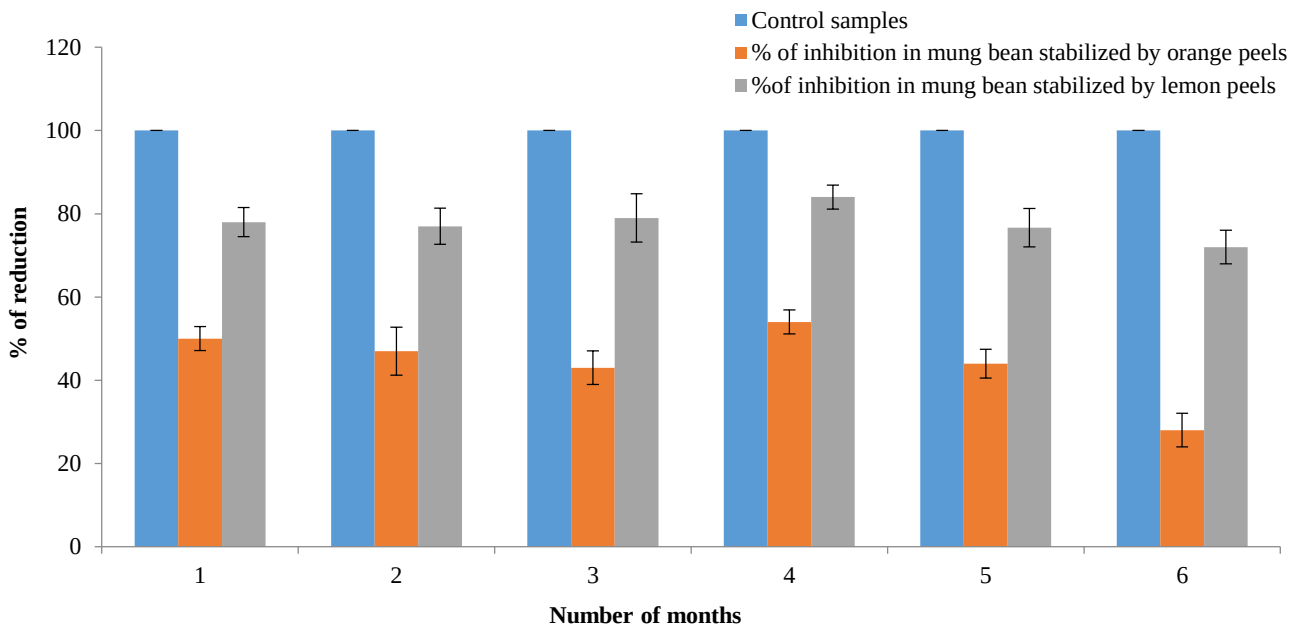

Fig. 4. Percentage % of aflatoxins reduction shown in chickpea stabilized by orange peels.

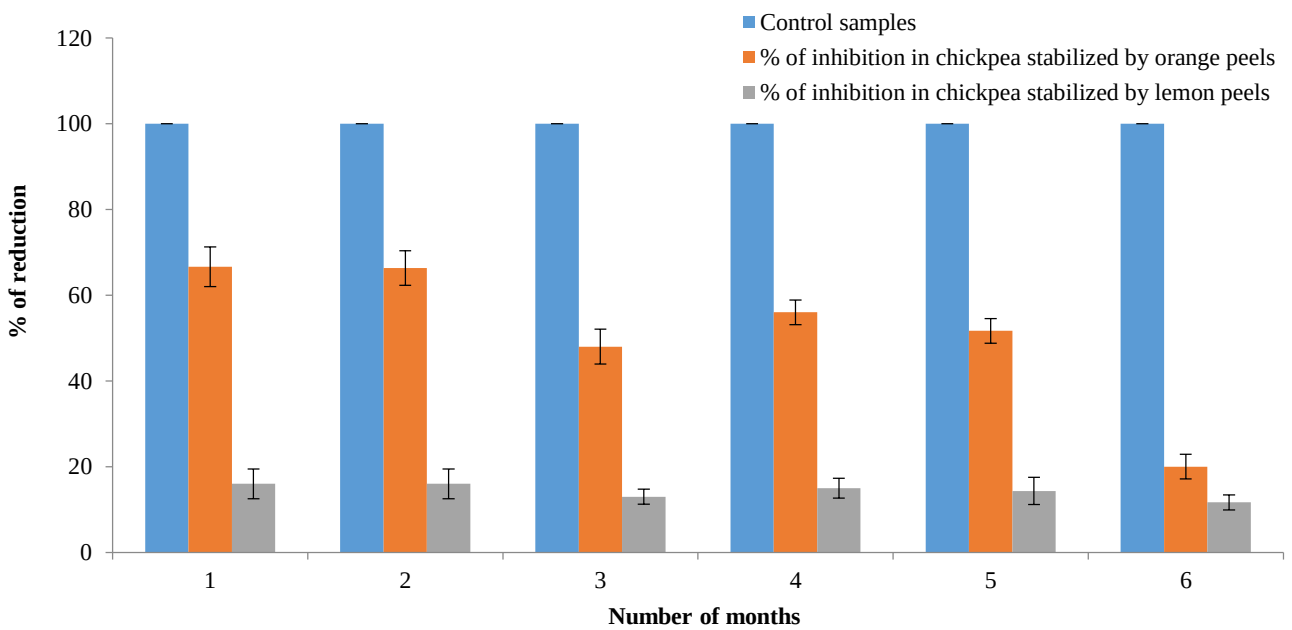

Fig. 5. Percentage % of aflatoxins reduction shown in mung bean stabilized by orange peels.

Conclusion and future perspectives: During this investigation, the inhibition of aflatoxins was done by orange and lemon peels powder in chickpea and mung bean and these peels showed positive results in reduction of aflatoxins produced by *Aspergillus* species. Concludingly, these peels can be presented in agroindustry advertise as a characteristic, protected and less expensive stabilizer substance to restrain aflatoxins generation during the storage of pulses. Major highlights of this research are:

- Micromycetes, especially *Aspergillus* spp. grew and produced aflatoxins in pulses (chickpea and mung bean)
- Orange and lemon peels substitutes chemical treatment by showing considerable antifungal activity
- Use of these peels is cost–effective and safer method for storage of pulses

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A: Appendix attached as supplementary materials.

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