World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

A preliminary study on biodetoxification of aflatoxins present in paddy straw and groundnut

V. Boopathi Raja, S. Chandrasekar* and V. Ramya

Department of Microbiology, Sri Krishna Arts and Science College, Coimbatore, Tamilnadu, India

Received: 28-07-2014 / Revised: 07-08-2014 / Accepted: 21-08-2014

ABSTRACT

Mycotoxins are fungal toxic metabolites which naturally contaminate food and feed. Aflatoxins are listed as Group I carcinogens by the International Agency for Research on Cancer (IARC), a body of the World Health Organization. Aflatoxins are one of the most important toxic metabolites which contaminates feed during storage. Consuming of this contaminated feed by the dairy cattle may result in decreasing milk yield, blindness, photosensitive dermatitis and some other reproductive problems. Various physical and chemical methods of reducing mycotoxins have been recommended, but only few have been accepted for practical use. In this study aflatoxin was extracted from contaminated paddy straw and groundnut with the help of The Association of analytical communities (AOAC) method and confirmed by High performance liquid chromatography (HPLC). Basidiomycetous fungus *Pleurotus sajor-caju* and laccase enzyme was used to degrade or transform aflatoxin into less toxic metabolites.

Keywords: Mycotoxins, *Pleurotus sajor-caju*, and laccase

INTRODUCTION

Mycotoxins are highly toxic fungal metabolites which have adverse effects on human and animal health. They are frequent contaminants of grain and fruit. Fungi and mycotoxins are potential problems for farmers and food producers because they can adversely affect production. Close attention should therefore be paid to the risk of contamination. Studies are required in order to understand the fate of these toxins during manufacturing, thereby improving risk management and reducing the potential for mycotoxin contamination [1].

Aflatoxins (AF) are mycotoxins derived by *Aspergillus flavus* and *Aspergillus parasiticus* are listed as Group I carcinogens by the International Agency for Research on Cancer (IARC), a body of the World Health Organization. The main aflatoxins are the B1 (AFB1), B2, G1 and G2 together with their metabolites, among which the most important is the aflatoxin B1. AFB1 is the molecule with the highest toxic significance. It is commonly found in any foodstuff or animal feed which can support fungal growth during growth, harvest, or storage [2]. Aflatoxins is typically found as secondary metabolites of *Aspergillus parasiticus and Aspergillus flavus* [3]. Epidemiological studies have shown that with prolonged exposure to AFB1 liver cancer may develop, especially in persons with hepatitis B antigens [4, 5]. It is commonly observed in animals include poor absorption of nutrients sometimes leading to death, reduced tissue integrity, lower growth rates and poor feed conversion, reduced immune response, reproductive problems in males and females, and increasing sensitivity to extreme temperatures [6, 7].

Biodegradation of aflatoxins, using microorganisms or enzymes, is one of the well-known strategies for the management of aflatoxins in foods and feeds. The methods of biodegradation are being actively studied and can be highly promising choice, since it is efficient, specific, and environmentally friendly to reduce or eliminate the possible contaminations of aflatoxins in foods and feeds [8].

This work is a preliminary attempt to detoxify the aflatoxin present in the Paddy straw and groundnut

***Corresponding Author Address:** Dr. S. Chandrasekar, Department of Microbiology, Sri Krishna Arts and Science College, Coimbatore, Tamilnadu, India; E-mail: sekar_biotech@yahoo.com*

with the help of laccase enzyme and *Pleurotus sajor-caju.*

MATERIALS AND METHOD

Collection of samples: Paddy straw and groundnut were collected from local market in Coimbatore and brought to laboratory using sterile container.

Preparation of Material: Paddy straw were cut into small pieces of approximately 2 cm length, washed with tap water and followed by distilled water to get rid of adhering particles. This was then allowed to dry at room temperature. While for groundnut, contaminated seeds were used for the extraction of aflatoxin.

Extraction of aflatoxin from contaminated groundnut and paddy straw: The Association of analytical communities (AOAC) method with some modifications was used for extraction of aflatoxin. Methanol and water in the ratio of 70: 30 was used as a solvent for aflatoxin extraction [9]. Aflatoxins were extracted out by using methanol as a solvent. 2 gm of the sample was taken and to this 70% methanol was added and ground it well using mortar and pestle. The extracted sample was stored at 4 °C. The same method was followed for groundnut extraction, where only naturally contaminated groundnut was used for extraction. It was stored in the refrigerator conditions (4 °C).

High Performance Liquid Chromatography: HPLC analysis was carried out at SynkroMax Biotech Pvt Ltd, Chennai, India. The aflatoxin B1 standard was obtained from SynkroMax. Working standard solution was prepared in acetonitrile water (1: 9, v/v) with concentration of 10 µg/ ml. The HPLC system used was Shimadzu LC-10 AT VP with fluorescence detection using a RF-10Ax1 detector. The eluate (20 µl) was injected under following conditions. A mobile phase of acetonitrile-methanol-water (300: 300: 600, v/v); a flow rate of 1 ml/ min. An excitation wavelength of 360 nm and an emission at 440 nm; a C-18 phenomenex column of 5 µm (250 x 4.5 nm); a total run time of 30 minutes. The post-column reagent pump flow rate was set at 0.15 ml/min under ambient temperature. The limit of quantification for aflatoxin B1 was 0.1 ppb.

Degradation of aflatoxin using *pleurotus sajor-caju* and laccase enzyme: For degradation, 1.5 ml of extraction of aflatoxin from contaminated groundnut and paddy straw, 1 ml of laccase enzyme was added and incubated for 24 hours at 37 °C. After incubation the same was measured at spectrophotometer at 330 nm. Similarly, it was done with *Pleurotus sajor-caju*.

RESULTS AND DISCUSSION

Preparation of a material: Paddy straw and groundnut seeds were collected from local market of Coimbatore and they were extracted by AOAC method and it was filtered using whatmann no.1 filter paper and stored at 4 °C. From the figure 1 it was found that the λ-max of standard control of aflatoxin is 1.321 in UV- Vis spectrophotometer at 330 nm. From the figure 2 it was found that the λ-max of extracted aflatoxin from paddy straw is 1.096 in UV- Vis spectrophotometer at 330 nm. From the figure 3 it was found that the λ-max of extracted aflatoxin from groundnut is 3.186 in UV-Vis spectrophotometer at 330 nm.

High performance liquid chromatography: AFB1 standard was identified as compound having mass-to-charge ratio (*m/z*) of 313.2 for the protonated cation $[M+H]^+$. This is in accordance with an earlier report which suggested the formation of protonated AFB1 cation. In addition, they also reported the formation of sodiated cation of AFB1 $[M+Na]^+$ HPLC analysis of the uninoculated substrate showed the occurrence of a compound, probably hydrated AFB1, with *m/z* of 331 .The occurrence of hydrated AFB1 could be attributed to the presence of abundant moisture within the solid substrate. Moreover, the presence of *Pleurotus sajor-caju* extract in the supplement solution as a source of laccase may explain the possible formation of AFB1-nitrogen adducts (*m/z* of 326.2). Under *in vivo* condition, AFB1 epoxide usually undergoes nucleophilic attack by the N7 nitrogen of deoxyguanosine in DNA. The formation of a compound with *m/z* of 355, probably AFB1-calcium adducted ions, may be correlated to the presence of $CaCO_3$ in the supplement solution.

Degradation of aflatoxin using laccase enzyme and *pleurotus sajor-caju*:

From the figure 5and 6 it was found that the λ-max of degraded aflatoxin from paddy straw and groundnut using *Pleurotus sajor-caju* is 0.109 and 0.232 in UV- Vis spectrophotometer at 330 nm respectively. Degradation of aflatoxin from paddy straw and groundnut using Laccase enzyme is 1.284 and 5.003 in UV- Vis spectrophotometer at 330 nm respectively(Fig 7 and 8). Aflatoxin produced by Aspergillus flavus is known to have carcinogenic and teratogenic effects on animal and human health. The basidiomycetous fungus *Pleurotus sajor-caju* can grown in different agronomic waste by synthesizing different lignolytic enzymes. These extracellular enzymes are capable of degrading many environmentally hazardous compounds including aflatoxin. The present study examines the ability to show the degrading level of aflatoxin in contaminated paddy

straw and groundnut using *Pleurotus sajor-caju* and laccase enzyme. From our findings using *Pleurotus sajor-caju* and laccase enzyme, the contaminated groundnut and paddy straw was degraded. Laccase enzyme has more efficient of degrading aflatoxin in contrast to *Pleurotus sajor-caju* (Table 1) (Fig 9). Even though *Pleurotus sajor-caju* produces laccase enzyme, synthesized form of laccase enzyme is more efficient in degrading aflatoxin. In a previous study, co-cultivation of *A. flavus* and *P. ostreatus* was carried out on various substrates. On wheat straw, corn cobs and millet, A. flavus produced aflatoxin after 3 weeks of cultivation. A subsequent cultivation of *P. ostreatus* on *A. flavus*-contaminated straw led to detoxification of the straw and corn cobs. It was found that *P. ostreatus* could liquidate colonies of *A. flavus*. However, cultivation of *P. ostreatus* in the presence of substrate did not result in complete detoxification of the material even after 34 days of co-cultivation, but AFB1 concentration decreased to about one-fourth of the added amount [10].

The present findings are significant as biodegradation of aflatoxin with microorganisms or their enzymes may be considered as the best strategy for detoxification of contaminated feedstuffs. This approach is considered as environment friendly in contrast to physico-chemical techniques of detoxification [11].

CONCLUSION

From the present study, it might be deduced that both *Pleurotus sajor-caju* and laccase enzyme degraded aflatoxin in contaminated paddy straw and groundnut. While comparing the degradation level of aflatoxin, laccase enzyme could effectively degrade than P*leurotus sajor-caju*. Further studies would be required to enhance the aflatoxin degradation potential of the basidiomycetes strains through the process optimization.

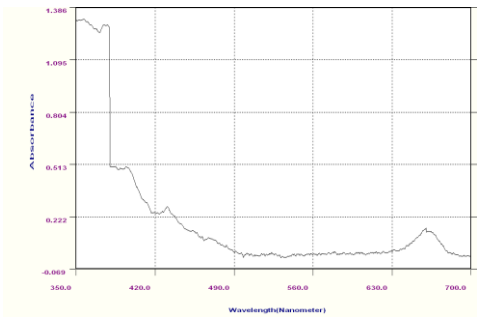
ACKNOWLEDGEMENTS

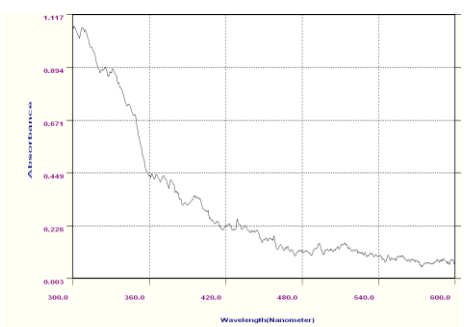
The authors are thankful to the management of Sri Krishna Arts and Science College (Autonomous), Coimbatore, for providing them necessary facilities and support to carry out this work. The authors are also thankful to R Raj Kumar - Biotechnologist, Centre for Bioscience and Nanoscience Research, Coimbatore for support to analyze the degradation of Aflatoxin.

Table 1: Absorbance values of degraded aflatoxin in paddy straw and groundnut by UV- Vis spectrophotometer at 330 nm

Sample taken for degrading Aflatoxin	Degradation of Aflatoxin present in Paddy straw	Degradation of Aflatoxin present in Groundnut
Pleurotus sajor-caju	0.109	0.232
Laccase enzyme	1.284	5.003

Figure 1 Figure 2 Figure 3





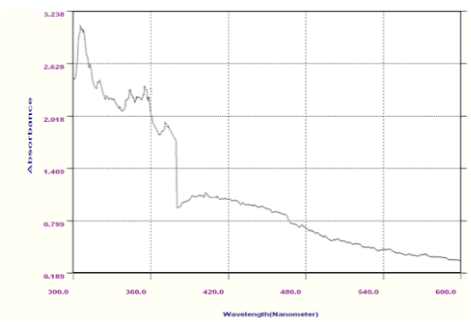
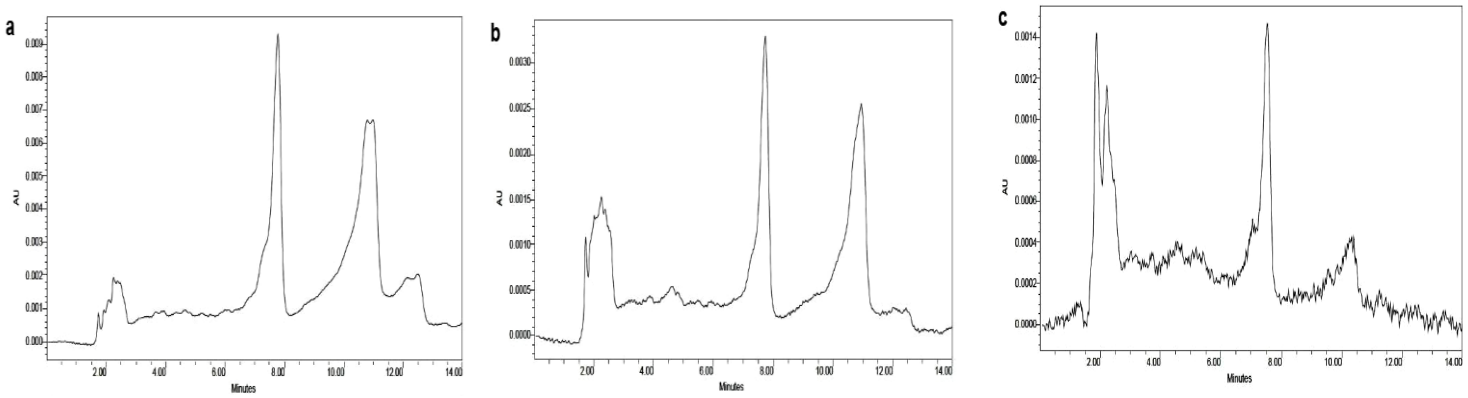


Figure 1; Absorbance of standard control of aflatoxin

Figure 2; Absorbance of extracted aflatoxin from Paddy straw

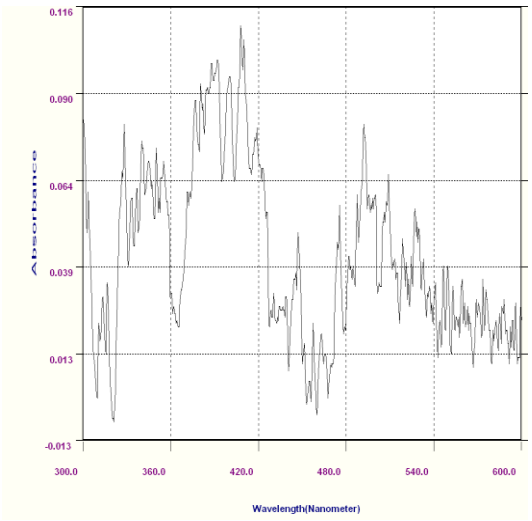
Figure 3; Absorbance of extracted aflatoxin from groundnut

Figure 4: HLPC chromatogram showing Aflatoxin peaks



a. Peak for control **b. Peak for Paddy straw** **C. Peak for groundnut**

Figure 5 Figure 6



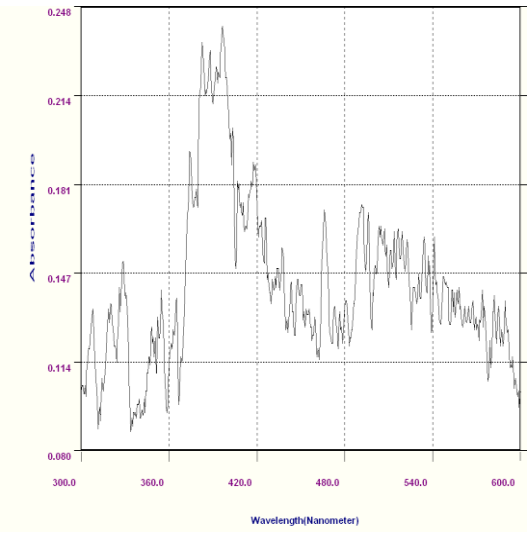


Figure 5: Absorbance of degraded aflatoxin in paddy straw using *Pleurotus sajorcaju*

Figure 6: Absorbance of degraded aflatoxin in groundnut using *Pleurotus sajorcaju*

Figure 7

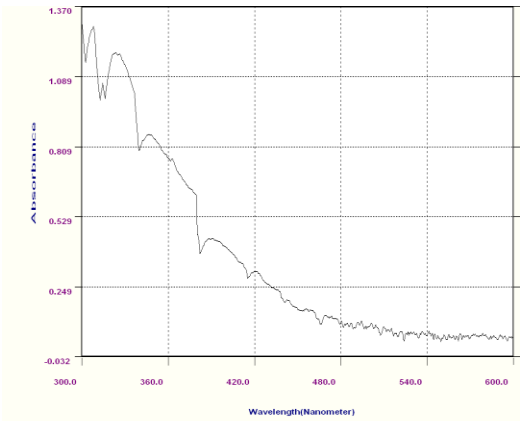


Figure 8

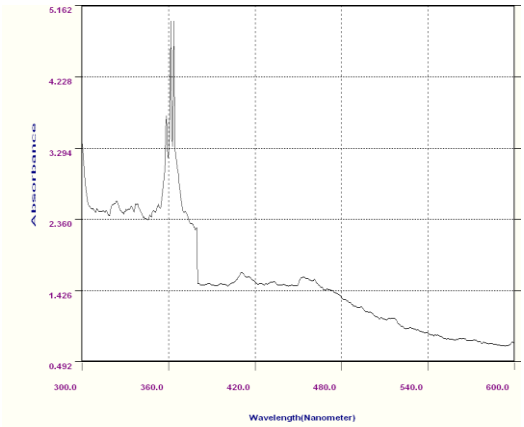
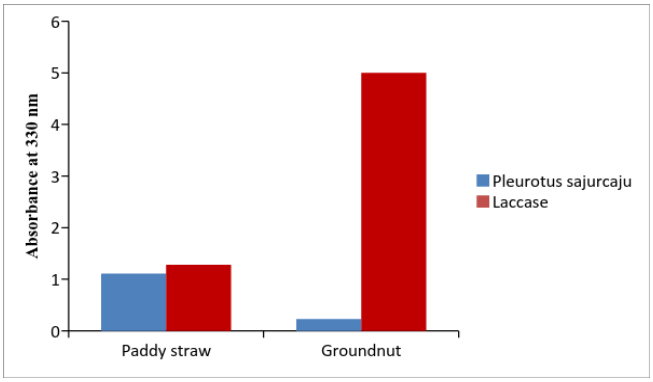


Figure 7: Absorbance of degraded aflatoxin in paddy straw using Laccase enzyme
Figure 8: Absorbance of degraded aflatoxin in groundnut using Laccase enzyme

Figure 9: Comparison of degrading level of *Pleurotus sajorcaju* and laccase enzyme in paddy straw and groundnut



REFERENCES

[1] Tomonori Inoue and Yasushi Nagatomi. Degradation of Aflatoxin B1 during the Fermentation of Alcoholic Beverages. Toxins. 2013:**5**:1219-1229.
[2] Shivender Singh Saini and Anupreet Kaur. Aflatoxin B1: Toxicity, characteristics and analysis.Global Advanced Research Journal of Chemistry and Material Science. 2012:1(4):63-70.
[3] Maragos CM, AE Pohland. Measurement of aflatoxins using capillary electrophoresis. In Mycotoxin Protocols, 1st Eds; Humana Press: Totowa, NJ.2001:51-58.
[4] Sun CA and CJ Chen. Aflatoxin-induced hepatocarcinogenesis. Epidemiological evidence and mechanistic considerations. Journal of Medicine Science. 2003: 23: 311-318.
[5] Lu FC Assessment of safety/risk vs. public health concerns: aflatoxins and hepatocarcinoma. Environmental Health Preventive Medicine. 2003:7: 235-238.
[6] Leeson S, G. Diaz, and J. D. Summer. Aflatoxins. Poultry Metabolic Disorders and Mycotoxins. University Books, Guelph, Canada. 1995: 249–298
[7] Davegowda G. and T. N. K. Murthy. Mycotoxins, their effects in poultry and some practical solutions. Mycotoxins Blue Book. D. E. Diaz, ed. Nottingham University Press, Nottingham, UK. 2005: 25– 56
[8] Qinghua Wu. Biological degradation of aflatoxins Drug Metabolism Reviews. 2009: 41:**1**: 1–7
[9] Whitaker. Official Methods of Analysis. AOAC, Arlington, VA. 14th Ed. 1984:**26**:026-26.048.
[10] Ginterova A, Polster M, Janotkova O. The relationship between *Pleurotus ostreatu*s and *Aspergillus flavus* and the production of aflatoxin. Folia Microbiol. 1980: 25:332–336
[11] Upadhaya SD, Park MA and Ha JK. Mycotoxins and their biotransformation in the rumen: a review. Asian Aust J Anim Sci. 2010:**23**:1250–1260.