Available Online through
www.ijpbs.com

IJPBS |Volume 2| Issue 2 |APRIL-JUNE |2012|94-105

Evaluation of Process Parameters Influencing Aflatoxin B1 Synthesis from Aspergillus flavus MTCC 2798 using Rice Straw under Submerged Fermentation

Arijit Das[1], J. Angayarkanni[2], Sourav Bhattacharya[3] and M. Palaniswamy[1]*

[1]*Department of Microbiology, Karpagam University, Coimbatore, Tamil Nadu, India*
[2]*Department of Microbial Biotechnology, Bharathiar University, Coimbatore, Tamil Nadu, India*
[3]*Department of Microbiology, Genohelix Biolabs,*
A Division of Centre for Advanced Studies in Biosciences, Jain University,
127/2, Bull Temple Road, Chamarajpet, Bangalore, Karnataka, India
**Corresponding Author Email: jit2007das@rediffmail.com*

BIOLOGICAL SCIENCES
RECEIVED ON 27-04-2012

RESEARCH ARTICLE
ACCEPTED ON 06-05-2012

ABSTRACT

Aflatoxin is a toxic secondary metabolite produced by different species of Aspergillus in food crops when stored under hot and humid conditions. Among the various aflatoxins produced by fungi aflatoxin B1 has been proved to have carcinogenic, mutagenic, teratogenic and immunosuppressive effects. They often contaminate human food, animal feed and agricultural produce. Rice straw acts as a commonly used cattle feed in India. Feeding of moldy straw may lead to aflatoxicosis in cattle and pose serious threat to dairy industry. The present study focuses on the evaluation of various nutritional and physical parameters that influence the synthesis of aflatoxin B1 from Aspergillus flavus MTCC 2798 in rice straw under submerged fermentation. The aflatoxin B1 production was detected by thin layer chromatography and its level was quantified by high- performance liquid chromatography. Among the various semi-synthetic media studied yeast extract sucrose medium supported the highest aflatoxin production (55.15 µg/ml). Static or stationary condition yielded higher level of aflatoxin than shaking condition. Among the nutritional parameters, 2% (w/v) rice straw and 0.5% (w/v) peptone supported maximum production of aflatoxin. The highest level of aflatoxin B1 was synthesized at pH 3.5 and at 30°C. An inoculum size of 5% (v/v) resulted in significant toxin production after 30 days of incubation under aerobic condition. It may be concluded that rice straw has the potential to support good production of aflatoxin when incubated under ambient conditions. Therefore, proper storage and periodical monitoring of its quality should be practised to prevent aflatoxicosis in cattle.

KEYWORDS: *Aspergillus flavus, aflatoxin B1, cattle feed, rice straw, submerged fermentation.*

INTRODUCTION

Mycotoxins are relatively high molecular weight fungal metabolites that contain one or more oxygenated alicyclic rings[1]. The mycotoxins of the greatest significance in foods and feeds are aflatoxins which have carcinogenic, mutagenic, teratogenic and immunosuppressive effects on the health of humans and animals[2, 3]. Prolonged intake of moderate to low concentrations of these mycotoxins may result in serious problems. Aflatoxins are a group of highly toxic secondary metabolites produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*[4, 5, 6]. They pose severe threat to both livestock productivity and human health, thus bringing huge worldwide economic losses each year[7].

Numerous reports suggest the hazardous nature of aflatoxins which lead to loss of productivity in farm animals consuming contaminated feeds and the carcinogenicity in experimental animals[8]. Consumption of aflatoxin-contaminated foods has also led to increase in the incidence of liver cancer in several populations in South East Asia and Africa[9].

Rice straw is widely used as cattle feed and once contaminated, may act as a potent cause of aflatoxicosis in cattle. A previous study reported the presence of *A. flavus* in 28% of

the examined samples of rice straw and hay[10]. Another study revealed that about 50% of the *A. flavus* isolates produced B1 and B2 aflatoxins in rice fodder[11]. Different environmental conditions, as well as agronomic, feed storage and feed handling practices favour the mold growth and aflatoxin formation[12]. Dampness also leads to the transmission of aflatoxin M from fermented straw to cow's milk in low level[13]. Therefore, the present investigation focuses on the effects of various nutritional and physical parameters that influence the production of aflatoxin B1 from a standard toxigenic strain of *A. flavus* in rice straw under submerged fermentation.

MATERIALS AND METHODS

The present study was conducted during the period from 18.03.2011 to 27.03.2012 at the Department of Microbiology, Genohelix Biolabs, Chamarajpet, Bangalore, India. The present investigation constitutes a part of Ph.D. research of the first author admitted to Karpagam University, Coimbatore, Tamil Nadu, India.

Chemicals and reagents:

All the media used during the course of the study were obtained from Himedia Laboratories Pvt. Limited (Mumbai, India). The analytical grade chemicals and reagents were purchased from Loba Chemie (Mumbai, India), Qualigens Fine Chemicals (Mumbai, India) and s d Fine-Chem Ltd. (Mumbai, India). All the other routine chemicals were procured from Nice Chemicals (Kochi, India). Pure distilled water was obtained with a Milli-Q system (Millipore, Tokyo, Japan). HPLC grade Aflatoxin B1 standard was procured from Sigma-Aldrich Co. (USA).

Source of fungal strain:

Aflatoxin producing standard strain of *Aspergillus flavus* MTCC 2798 was obtained from Microbial Type Culture Collection, Chandigarh, India. The fungus was propagated on potato dextrose agar slants and the colony morphology and microscopic features were also noted. Pure culture was maintained at 4°C until use.

Detection of aflatoxin production:

Spore suspension of *A. flavus* MTCC 2798 was aseptically inoculated into sterile Czapek Dox broth and incubated under static condition at 28°C and also under shaking at 130 rpm for 15 days. The mold broth was filtered through normal filter paper and then through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, England) and the filtrate was collected. To the filtrate equal volume of chloroform was added and the contents were shaken for 30 min. The chloroform fraction was collected using a separating funnel. The presence of aflatoxin was detected using thin layer chromatography consisting of silica gel plates (Merck Ltd., Mumbai, India) as the stationary phase and a mixture of ether-methanol-water (96: 3: 1, v/v) as the mobile phase. Aflatoxin B1 was confirmed by spraying the thin layer chromatographic plates with aqueous sulphuric acid (50:50, v/v), dried and the fluorescence was visualized under UV light using a gel documentation unit (Herolabs, Germany)[14].

Extraction and partition of aflatoxin:

The extraction, purification and derivatization of aflatoxin were performed in accordance with the standard protocols[15]. The mycelial broth was taken into a blender jar and 100 ml of methanol was added, followed by 25 ml of 0.1 N HCl. The contents were blended for 3 min at high speed and filtered through Whatman No. 1 filter paper. 50 ml of the filtrate was collected and transferred to a separating funnel and 50 ml of 10% (w/v) NaCl solution was added and swirled for 30 sec. To

this, 50 ml of hexane was added and gently shaken for 30 sec. The phases were allowed to separate and the lower aqueous phase was taken into another separating funnel. To this aqueous layer, 25 ml of methylene chloride was added and moderately shaken for 30 sec. When the phases separated, the lower methylene chloride layer was transferred to another separating funnel and the partitioning was repeated twice with methylene chloride. The resultant methylene chloride fraction was evaporated to 2-3 ml at 40°C in a hot air oven.

Purification of aflatoxin using silica gel column chromatography:

A slurry of 2 g of silica gel was prepared with 10 ml ether-hexane (3:1, v/v) and packed into the column. The gel was allowed to settle without tamping. After the gel settled, the column was drained and 1 g of sodium sulphate was added over the silica. 5-6 ml of methylene chloride fraction of aflatoxin was added. The column was washed with 25 ml of benzene-acetic acid (9:1, v/v), followed by 30 ml of ether-hexane (3:1, v/v). Aflatoxin was eluted with 50 ml of methylene chloride-acetone (9:1, v/v). The eluate was evaporated to 0.5 ml at 40°C in a hot air oven.

Derivatization of aflatoxin:

0.5 ml of aflatoxin fraction was evaporated to dryness. 0.2 ml of hexane and 50 µl of trifluoroacetic acid were added to it and vortexed for 30 sec. After 5 min, 1 ml of water-acetonitrile (9:1, v/v) was added and vortexed for 30 sec. After the phases separated, 20 µl of the lower aqueous layer was injected into the HPLC column.

Quantitation of aflatoxin using High-Performance Liquid Chromatography (HPLC):

The HPLC analysis of aflatoxin was carried out at IADFAC Laboratories Pvt. Ltd., Bangalore, India. The aflatoxin B1 standard was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The 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-10A_{XI} detector. The eluate (20 µl) was injected under the following conditions: a mobile phase of acetonitrile-methanol-water (300:300:600, v/v); a flow rate of 1 ml min^{-1}; an excitation wavelength of 360 nm and an emission at 440 nm; a C-18 Phenomenex column of 5 µm (250 x 4.5 mm); a total run time of 30 min. The post-column reagent pump flow rate was set at 0.15 ml min^{-1} under ambient temperature. The limit of quantification for aflatoxin B1 was 0.1 ppb.

The concentration of aflatoxin B1 stock standard solution (about 1 mg/100 ml) in a mixture of benzene and acetonitrile (98:2, v/v) was determined by UV spectroscopy according to the following equation:

Concentration of aflatoxin (µg/ml)

$$= (A_{350} \times M_w \times 1000)/\varepsilon$$

Where,

A_{350} = the absorbance of aflatoxin B1 at a wavelength of maximum absorption close to 350 nm,

M_w = the molecular weight of aflatoxin B1 (312),

ε = the molar absorptivity of aflatoxin B1 in benzene-acetonitrile solution (98:2, v/v) (19800).

Aflatoxin production associated with rice straw:

Various semi-synthetic media such as yeast extract sucrose medium, sucrose magnesium potassium yeast extract medium and Czapek Dox + yeast extract medium were screened to study the aflatoxin B1 production by *A. flavus* MTCC 2798. The best medium was selected and its carbon source was substituted with rice straw. The effects of various process parameters influencing the moldiness and aflatoxin production in rice straw such as types

and percentages of nutritional supplements, pH of media, temperature, inoculum size and incubation time were investigated during this study in submerged fermentation (SmF).

Source of substrate:

Rice straw was procured from the local market in Bangalore city. The hay was chopped into small pieces, washed several times with clean water to get rid of visible dirt and kept for drying. This was utilized as the substrate (carbon source) for aflatoxin production.

Influence of nutritional supplements:

The effect of substrate percentage was examined by incorporating varying concentrations (0.05, 1, 2, 5 and 10% w/v) of rice straw. The effects of different organic (yeast extract, beef extract, peptone, tryptone, soybean meal, glycine and urea) and inorganic nitrogen sources (ammonium nitrate, ammonium chloride and sodium nitrite) at varying percentages (0, 0.05, 0.1, 0.25, 0.5, 1, 2, 3, 5, 7 and 10% w/v) were determined.

Influence of physical parameters:

The role of pH was studied by adjusting the media pH from 3 to 9 with a gradual increment of 0.5 pH unit. Production of aflatoxin was noted at 4, 25, 30, 37 and 42°C. The influence of inoculum size was investigated using various percentages (0.5, 1, 3, 5 and 10% v/v). The effect of incubation time on aflatoxin production was determined after every 5 days for 30 days.

Statistical analysis:

All the studies were conducted in triplicates and the data were analyzed using single factor analysis of variance (ANOVA). All the data are graphically presented as the mean ± standard deviation of triplicates (n = 3). ANOVA was performed using Microsoft Excel 2007. *p* values<0.05 were considered significant with a confidence limit of 95%.

RESULTS AND DISCUSSION

Among the various mycotoxins produced by fungi, aflatoxins are significantly important as they pose potential threat to agriculture and food processing industries. Aflatoxins are toxic secondary metabolites produced by species of *Aspergillus* in cereals, grains and other food crops both in field as well as under storage conditions. Aflatoxin B1 is considered to be most toxic among all the aflatoxins owing to its hepatocarcinogenic and other detrimental effects. The occurrence of aflatoxin B1 in animal feed, forage and agricultural produce may result in aflatoxicosis in cattle. There is evidence that aflatoxin present in the cattle feed may be transmitted to cow's milk and eventually to human beings consuming the milk[16].

Rice straw is generally contaminated by mycotoxigenic strains of *Aspergillus, Penicillium, Fusarium, Alternaria, Stachybotrys* and *Cladosporium*[17, 18, 19]. Since the production of rice straw is seasonal, the farmers traditionally store it for several months to feed their animals. Therefore, it is very likely that the moldy straw could be contaminated with mycotoxin[20]. Moreover, the presence of 85.94-87.71% organic matter and 4.06-4.12% crude protein in the rice straw may render it suitable for aflatoxin production[13, 21].

Aflatoxigenic fungal strain:

The aflatoxin B1 producing fungal strain *A. flavus* MTCC 2798 obtained from MTCC, Chandigarh, India was propagated on potato dextrose agar, wherein it produced the characteristic yellowish green powdery colonies. Microscopic observation revealed the presence of chains of green spherical conidia arranged on globose vesicles at the end of erect conidiophores.

Detection of aflatoxin production:

For the initial detection of aflatoxin production the mold strain was grown in a synthetic

medium Czapek Dox broth and incubated at 28°C for 15 days under both static and shaker conditions. With an increase in the incubation time beyond 7 days, the medium developed a yellowish orange hue probably due to the production of secondary metabolite. Following the fungal growth, aflatoxin was extracted with chloroform and subjected to TLC for qualitative detection. The TLC plates when exposed to UV light showed characteristic blue fluorescence indicating the presence of aflatoxin B in the sample.

Extraction, partition and purification of aflatoxin:

The extraction and partition of aflatoxin from the aqueous broth filtrate were performed using the solvent methylene chloride or dichloromethane. This was followed by purification of the methylene chloride fraction using silica gel column chromatography. Prior to HPLC analysis and quantification the purified aflatoxin was also derivatized. These extensive sample preparations increased the concentration of aflatoxin in the final sample than initially present in the broth filtrate.

Quantitation of aflatoxin using HPLC:

The level of aflatoxin in the sample was quantified using HPLC with a fluorescence detector. The amounts of aflatoxin B1 produced by *A. flavus* MTCC 2798 were evaluated as 9.2 µg/ml and 7.54 µg/ml under static and shaking conditions, respectively. The higher production of aflatoxin under static condition might be attributed to the faster depletion of nutrients and oxygen content due to the production of a thick fungal mat over the broth surface. This would have resulted in a stationary phase faster than the shaking condition, thereby resulting in increased level of aflatoxin production. Earlier workers also reported that 35% of *A. flavus* produced amounts of AFB1 that ranged from 1-100 µg per 25 ml of YES medium while only 2% could produce more than 200 µg AFB1 per 25 ml of YES medium[10].

Nutritional parameters affecting aflatoxin production:

Among the various semi-synthetic media used, maximum aflatoxin production was detected in yeast extract sucrose medium with a level of 55.15 µg/ml **(Figure 1)**. Similar result was reported in a previous study where the yeast extract sucrose medium supplemented with sorbic acid or p- cresol supported good production of aflatoxin B1 by standard toxigenic strain of *A. flavus*[10]. In general, YES medium is widely used to test toxin production in fungi belonging to *Penicillium* and *Aspergillus* species[22]. This may be due to the presence of very high content of carbon (sucrose) and nitrogen (yeast extract) in the yeast extract sucrose medium which has supported the maximum toxin synthesis. The least degree of production was recorded with Czapek Dox + yeast extract medium with a value of 15.17 µg/ml.

The carbon source of the yeast extract sucrose medium (sucrose) was substituted with rice straw to study the effect of various nutritional parameters on the aflatoxin biosynthesis. When the rice straw was incorporated at varying percentages into the production medium, 2% (w/v) straw revealed highest level of aflatoxin production with a value of 6.52 µg/ml. A further increase in the straw percentage led to reduction in the toxin production **(Figure 2)**.

Addition of nitrogen supplements into the production medium often enhances the metabolite production. In the present study the effects of different organic and inorganic nitrogen supplements were investigated. Among the organic nitrogen sources, peptone yielded the highest aflatoxin production (55.51 µg/ml) followed by beef extract (32.46 µg/ml) and soyabean meal (32.39 µg/ml) **(Figure 3)**.

This may be due to the occurrence of high amounts of partially digested proteins, proteoses and amino acids in peptone which favoured the aflatoxin biosynthesis. The other organic nitrogen sources exhibited moderate level of aflatoxin production. On the other hand, ammonium chloride, as the inorganic nitrogen supplement, showed the highest toxin synthesis (12.6 µg/ml). Based on these results, when the percentage of peptone was varied, 0.5% (w/v) peptone favoured the highest aflatoxin production (27.63 µg/ml) **(Figure 4)**.

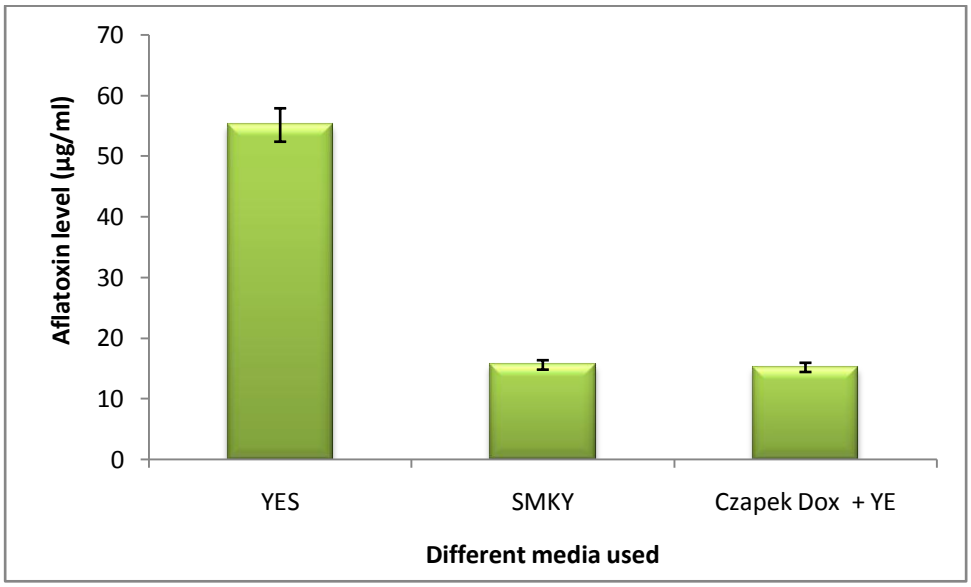

Figure 1: Effect of different media on the level of aflatoxin production from *A. flavus* MTCC 2798 in SmF. Data represent mean ± S.D. (n=3); *P* < 0.05. YES, yeast extract sucrose medium; SMKY, sucrose magnesium potassium yeast extract medium; Czapek Dox + YE, Czapek Dox + yeast extract medium.

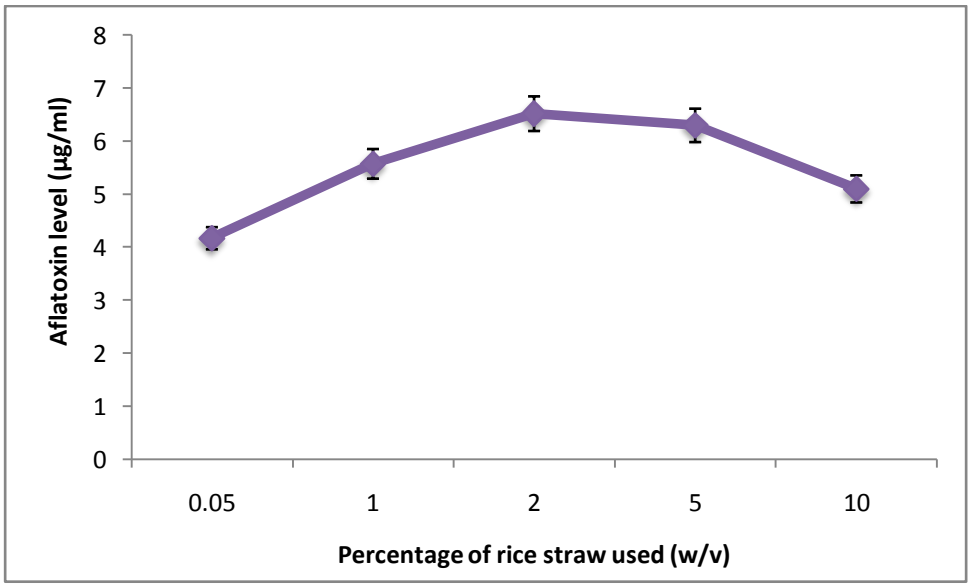

Figure 2: Effect of percentages of rice straw on aflatoxin production from *A. flavus* MTCC 2798 in SmF. Data represent mean ± S.D. (n=3); *P* < 0.05

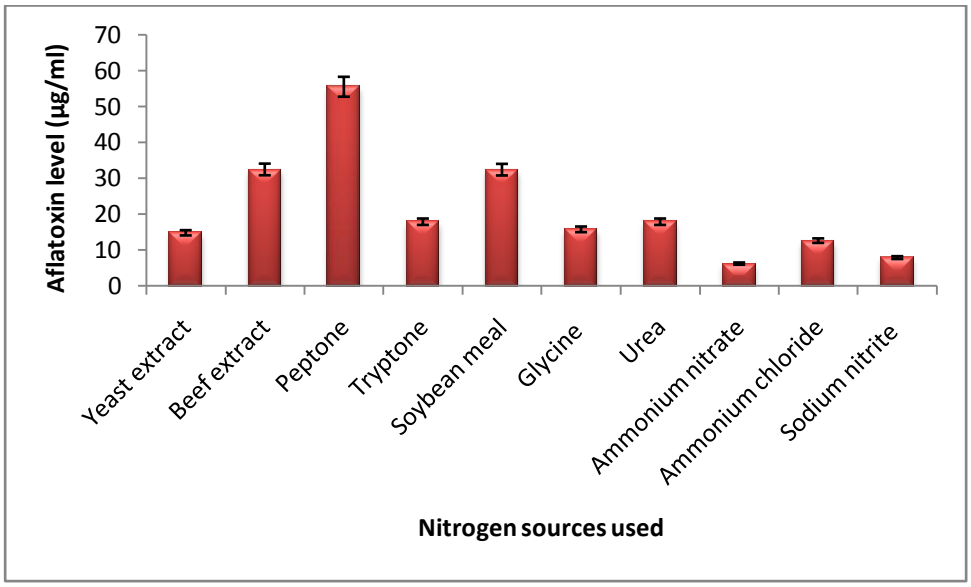

Figure 3: Effect of different nitrogen supplements on aflatoxin production from *A. flavus* MTCC 2798 in SmF. Data represent mean ± S.D. (n=3); *P* < 0.05

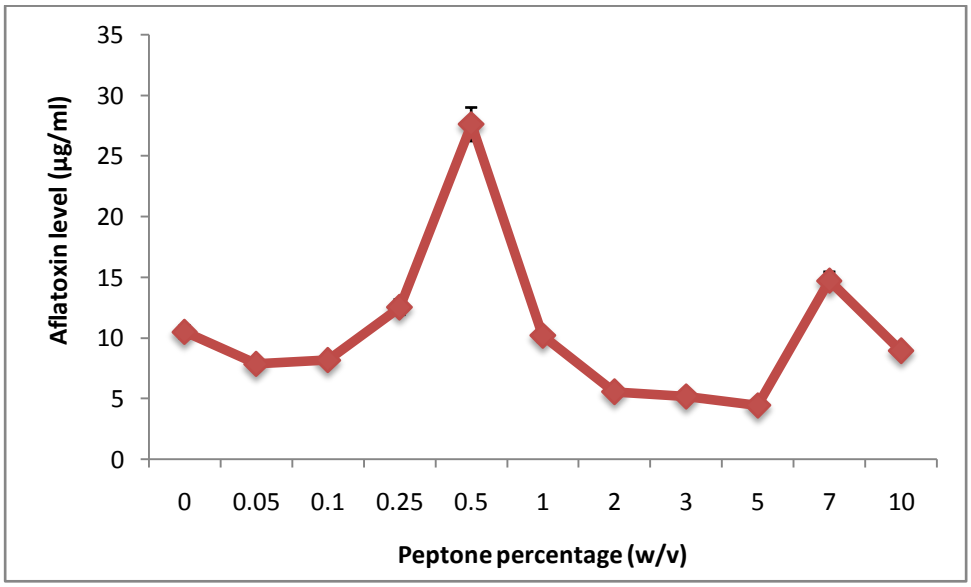

Figure 4: Effect of percentages of nitrogen supplement on aflatoxin production from *A. flavus* MTCC 2798 in SmF. Data represent mean ± S.D. (n=3); *P* < 0.05

Role of physical parameters on aflatoxin synthesis:

The pH of the production medium plays a vital role in the synthesis and accumulation of primary and secondary metabolites in the medium. Any change in the pH leads to altered level of the metabolite production. The pH of the plain rice straw was found to be between 7.4-7.5. To study the effect of pH on aflatoxin biosynthesis the media pH was adjusted after adding the rice straw into the production medium. Across the wide range of pH studied,

pH 3.5 was found to support the highest level of aflatoxin production (58.28 µg/ml) **(Figure 5)**. A previous study had reported the production of aflatoxin B in the acidic pH range, whereas, aflatoxin G was found to be synthesized in the alkaline pH range. The present findings are in accordance with this previous report as aflatoxin B1 synthesis was found to occur in the acidic pH range between pH 3-pH 7. Beyond pH 7.5, the level of aflatoxin B1 has found to be drastically reduced. On the contrary, a low pH of the medium reduced the synthesis of gliotoxin from *A. fumigatus,* suggesting that acid-stored feeds are less likely to have deleterious amounts of this toxin, even in the presence of *A. fumigatus* contamination[23].

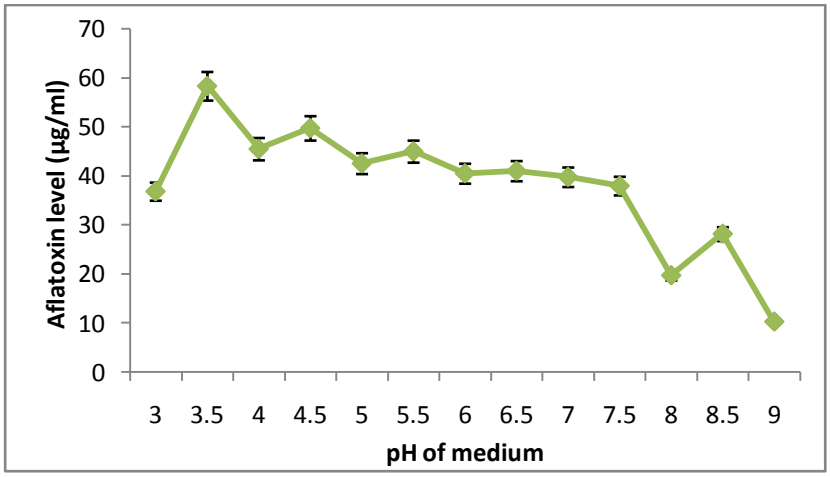

Figure 5: Effect of different pH on aflatoxin production from *A. flavus* MTCC 2798 in SmF. Data represents mean ± S.D. (n=3); *P* < 0.05

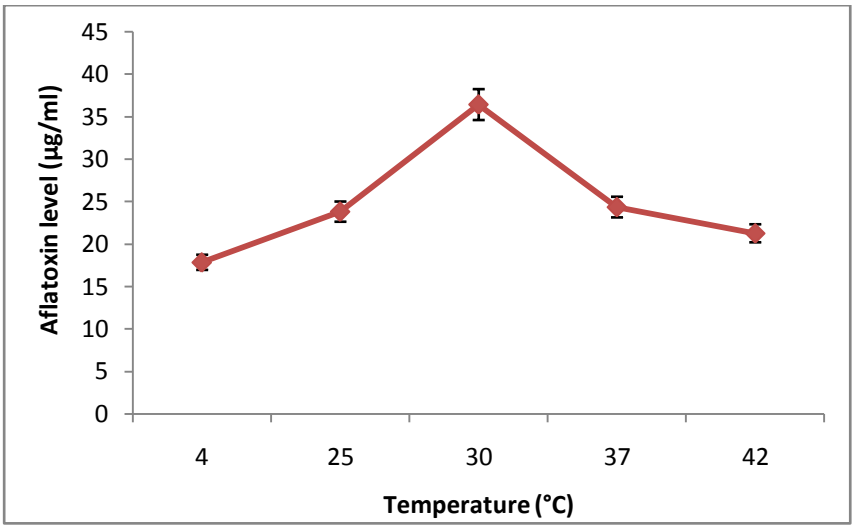

Figure 6: Effect of different temperatures on aflatoxin production from *A. flavus* MTCC 2798 in SmF. Data represents mean ± S.D. (n=3); *P* < 0.05

Environmental factor such as temperature also greatly influences the growth of the mold and subsequently the production of aflatoxin[24]. Generally, aflatoxin is synthesized when food grains and forage are stored under conditions of high temperature and high relative

humidity. In our study 30°C exhibited the highest level of aflatoxin production (36.4 µg/ml) **(Figure 6)**. *A. flavus* is a mesophilic mold and thereby contaminate food crops with aflatoxin when they are stored at around 30°C. An ambient temperature around 30°C was also found to support good sporulation of the mold. Our result is in accordance with the report suggesting that 30°C is best suited for aflatoxin production from *A.* flavus[25]. Low temperature of 4°C and relatively high temperature of 42°C demonstrated decreased production of aflatoxin.

The load of contaminating microorganisms acts as a determining factor for rapid spoilage of food and accumulation of toxins in it. When the inoculum size was varied, an increase in the toxin production was noted with an increase in spore suspension. 5% (v/v) spore suspension resulted in the highest production of aflatoxin (33.45 µg/ml) **(Figure 7)**. This might be due to greater biomass synthesis resulting in more rapid utilization of the rice straw and depletion of nutrients, thereby leading to enhanced aflatoxin production. Further increase in the inoculum size would have led to decrease in the oxygen potential of the medium, simultaneously affecting the fungal growth and aflatoxin production. On the other hand, reduced size of the inoculum had shown a negative effect on gliotoxin production from *A. fumigatus*[23].

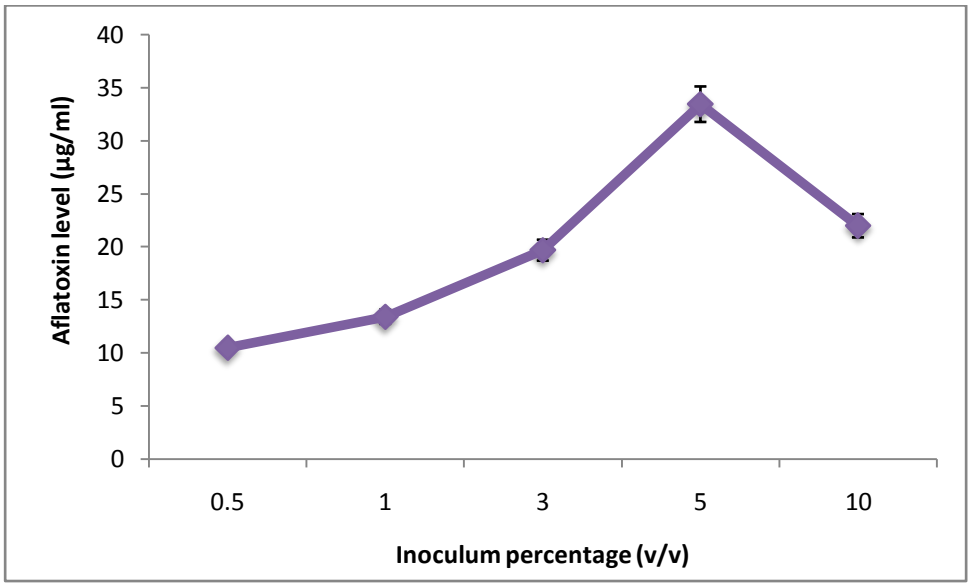

Figure 7: Effect of inoculum percentages on aflatoxin production from *A. flavus* MTCC 2798 in SmF. Data represents mean ± S.D. (n=3); *P* < 0.05

The production of any secondary metabolite usually occurs during the late stationary phase in the microbial growth cycle. Similar observation was made in the present study wherein the level of aflatoxin was found to be enhanced with an increase in the incubation time. This result is in agreement with a previous report suggesting that the concentration of verruculogen, fumagillin and helvolic acid in *A. fumigatus* cultures increased throughout the incubation period[22]. Extensive fungal growth was observed in rice straw substrate after 5-6 days of incubation. A highest level of toxin production (40.07 µg/ml) was obtained after 30 days of incubation under aerobic condition **(Figure 8)**. A previous study also suggests a significant level of aflatoxin B1 production (287.5 ppb) after 20 days of

incubation[10].The growth of the mold under submerged fermentation condition might also have been facilitated by the presence of abundant relative humidity over the entire incubation duration of 30 days. Aflatoxin being a stable metabolite remains accumulated in the medium over a long period of time. Under the natural storage conditions the produced aflatoxin may persist for a long time in the damp rice straw[13]. Similarly, in a study conducted previously on sorghum, aflatoxin B1 level was found to be significantly higher especially during the rainy season[14].

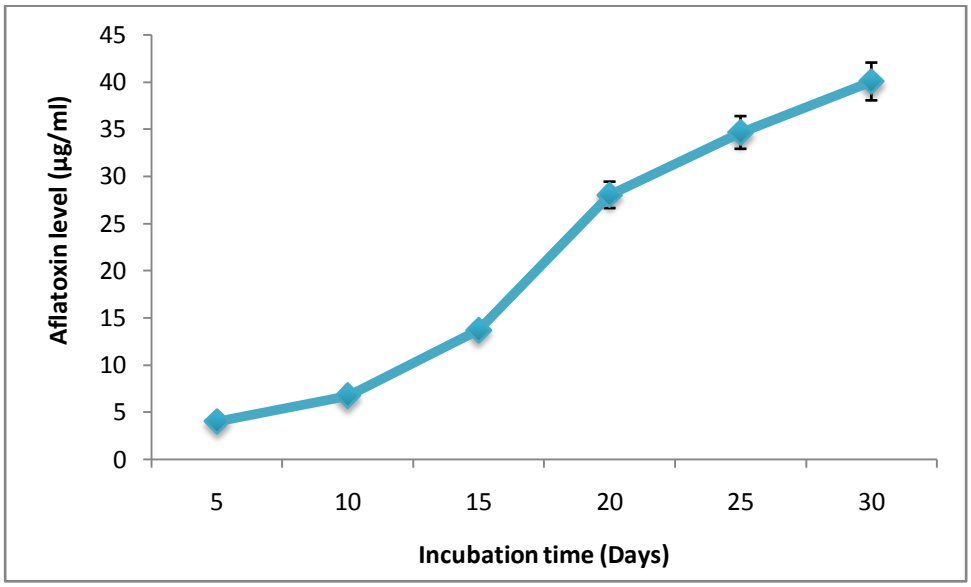

Figure 8: Effect of incubation time on aflatoxin production from *A. flavus* MTCC 2798 in SmF. Data represents mean ± S.D. (n=3); *P* < 0.05

CONCLUSION

The study suggests that various cultural and environmental factors affect the production of aflatoxin B1 in rice straw. The level of toxin production may vary from moderate to high, making the toxin-contaminated rice straw unsuitable and hazardous as cattle feed. Therefore, rice straw needs to be properly harvested, handled and stored under hygienic conditions to reduce the chance of aflatoxin contamination. Frequent monitoring of its quality should also be practised to prevent incidences of aflatoxicosis in cattle.

ACKNOWLEDGEMENTS

We wish to extend our sincere gratitude to the managements of Karpagam University, Jain University and Bharathiar University for their encouraging support. Our special thanks to Dr. R. Chenraj Jain, Chairman, Jain Group of Institutions, Bangalore, for providing us with the laboratory facilities required for this research work. We also wish to thank the entire supporting staff of the laboratory whose help has been invaluable for the successful completion of our research work.

REFERENCES

1. Primer, A., 2001. HPLC for Food Analysis. Agilent Technologies Company, Germany: 21-23.
2. Eaton, D.L. and Gallagher, E.P., 1994. Mechanisms of aflatoxin carcinogenesis, *Annu. Rev. Pharmacol. Toxicol., 34*: 135-172.
3. Jouany, J.P., Yiannikouris, A. and Bertin, G., 2005. The chemical bonds between mycotoxins and cell wall components of *Saccharomyces cerevisiae* have been identified, *Archiva Zootechnica, 8*: 26- 50.

4. Cary, J.W., Linz, J.E. and Bhatnagar, D., 2000. Aflatoxins: Biological Significance and Regulation of Biosynthesis. In: Microbial Foodborne Diseases: Mechanisms of Pathogenesis and Toxin Synthesis. Edited by Cary, J.W., Linz, J.E. and Bhatnagar, D. Technomic Publishing Corp. Lancaster, Pennsylvania: 317-361.
5. CAST (Council for Agricultural Science and Technology), 2002. Mycotoxins: Risks in Plant Animal and Human Systems. Ames, Iowa: CAST: 199.
6. Bhatnagar, D., Ehrlich, K.C. and Cleveland, T.E., 2003. Molecular genetic analysis and regulation of aflatoxin biosynthesis, *Appl. Microbiol. Biotechnol., 61*: 83-93.
7. Diaz, D.E., 2005. The Mycotoxin Blue Book. Nottingham University Press. Nottingham, England.
8. Palmgren, M.S. and Hayes, A.W., 1987. Aflatoxins in Foods: Mycotoxins in Food, Pallenkrogh Academic Press, London: 65-96.
9. Peers, F.G. and Linsell, C.A., 1973. Dietary aflatoxin and liver cancer- a population based study in Kenya, *Br. J. Cancer, 27*: 473-484.
10. Azab, R.M., Tawakkol, W.M., Hamad, A.M., Abou-Elmagd, M.K., El-Agrab, H.M. and Refai, M.K., 2005. Detection and estimation of aflatoxin B1 in feeds and its biodegradation by bacteria and fungi, *Egy. J. Nat. Toxins, 2*: 39-56.
11. Surekha, M., Saini, K., Reddy, V.K., Reddy, A.R. and Reddy, S.M., 2011. Fungal succession in stored rice (*Oryza sativa* Linn.) fodder and mycotoxin production, *Afr. J. Biotechnol., 10* (4): 550-555.
12. Whitlow, L.W. and Hagler, Jr W.M., 2002. Mycotoxins in feeds. *Feedstuffs, 1072*: 68-78.
13. Bhuiyan, A., Akbar, M.A. and Hossain, M.E., 2003. Nutritive value of damp rice straw and its feeding effect on aflatoxin transmission into cows milk, *Pak. J. Nutr., 2*(3): 153-158.
14. Hussaini, A.M., Timothy, A.G., Olufunmilayo, H.A., Ezekiel, A.S. and Godwin, H.O., 2009. Fungi and some mycotoxins found in mouldy sorghum in Niger State, Nigeria, *World J. Agric. Sci., 5* (1): 5-17.
15. Association of Official Analytical Chemists International (AOAC), 2000. Official Methods of Analysis of AOAC International (17th ed.). AOAC Int., Gaithersburg, MD.
16. Coker, R.D., 1999. Mycotoxins: The silent threat to human and animal health. Inaugural Lecture Series, The University of Greenwich, UK.
17. Phillips, S. and Wareing, P., 1993. Mycological analysis of rice straw samples collected in Bangladesh. File Note, Project AO317. Natural Resources Institute, Chatham Maritime, UK.
18. Phillips, S.I., Wareing, P.W., Dutta, A., Panigrahi, S. and Medlock, V., 1996. The mycoflora and incidence of mycotoxin, zearalenone and sterigmatocystin in dairy feed and forage samples from Eastern India and Bangladesh, *Mycopathologia, 5:* 1-7.
19. Reddy, K.R.N., Reddy, C.S. and Muralidharan, K., 2009. Detection of *Aspergillus* spp. and aflatoxins B1 in rice in India, *Food Microbiol., 26*: 27-31.
20. Coker, R.D., 1979. Aflatoxin: past, present and future. *Trop. Sci., 21*: 143-162.
21. Al-Mamun, M., Akbar, M.A. and Shahjalal, M., 2002. Rice straw, its quality and quantity as affected by storage systems in Bangladesh, *Pak. J. Nutr., 1*(3): 153-155.
22. Bayman, P., Baker, J.L., Doster, M.A., Michailides, T.J. and Mahoney, N.E., 2002. Ochratoxin production by the *Aspergillus ochraceus* group and *Aspergillus alliaceus*, *Appl. Environ. Microbiol., 68*: 2326–2329.
23. Boudra, H. and Morgavi, D.P., 2005. Mycotoxin risk evaluation in feeds contaminated by *Aspergillus fumigatus, Anim. Feed Sci. Technol., 120*: 113–123.
24. Maragos, C.M., 2008. Extraction of aflatoxins B1 and G1 from maize by using aqueous sodium dodecyl sulphate, *J. AOAC Int., 91*(4): 762-767.
25. Russell R., Paterson, M. and Lima, N., 2010. How will climate change affect mycotoxins in food? *Food Res. Int., 43*: 1902–1914.

***Corresponding Author:**
Arijit Das
Department of Microbiology,
Karpagam University, Coimbatore, Tamil Nadu,
India.
Phone: +919886919207.
E-mail: jit2007das@rediffmail.com