Fungal Toxicity Control in Oryza Sativa (basmati rice) using Climatic and Storage Conditions

Muhammad Asif Asghar[1], Farman Ahmed[1], SuraiyaJabeen[2]*, HibaAsif[2], KanwalHussain,[2] MuqaddusUsman[2]

[1]Food and Feed Safety Laboratory, Food and Marine Resources Research Centre, PCSIR Laboratories Complex Karachi - 75280 Pakistan

[2]Institute of Environmental studies, University of Karachi- 75270 Pakistan

*E-mail: masif345@yahoo.com

ABSTRACT

Introduction: Aflatoxins (AFs) are known carcinogenic, hepatotoxic and mutagenic which are produced by Aspergillus fungal species, directly or indirectly infect the humans and animals health through contaminated food and feed (Tahir et al., 2018). AFs have been found in a variety of foods including cereals, nuts and grains. However, the climatic conditions are directly impact on the AFs contamination levels (Norlia et al., 2020). For the prevention, effective control, and management of aflatoxins in food is required. Many effective physical, chemical, biological and genetic engineering techniques have been used to control the AFs (Martins et al., 2017). The most emerging technique for preventative measures of AFs is hermetic storage (Odjo et al., 2020). Hermetic storage is defined as airtight conditions that suppress the growth of fungal growth and AFs production.

Objectives: This research provides the effect of different temperatures and moisture contents (MC) on the *Aspergillus* growth and AFB_1 production in hermetic and non-hermetic conditions. This study also determines the effects of environmental factors and storage technique on Afs production in Basmati rice (OryzaSativa) during incubation period. It evaluates the strategies for pre-harvest phases and preservations for food packaging from toxins production.

Methodology: Initially fungal spores were isolated from sesame seeds using peptone water and incubated on PDA at 30°C. After 5 days of incubation, dark green colonies of *Aspergillus parasiticus* were appeared which was isolated grown on PDA containing plate. The toxigenicity of strain was confirmed using the PCR technique. In addition, the strain was also tested for AFs production on Czapekdox agar medium as described by Alshannaqet al 2020.The spore suspension was prepared in 0.05% Tween 80 aqueous solution and adjusted to 0.25 (OD540 nm) which contained approximately 106–7 conidia/mL. Spores count in the inoculum was verified using an automated cell counter (Yogendrarajahet al.,2016). Basmati rice sample was purchased from retail market of Karachi city. Initial MC of sample was determined using hot air oven and adjusted to provide control environment for AFs production. Moreover, isolated Aspergillus fungal spores comprising (1×106spore/mL) were spiked into rice samples. Petri plates were Incubated for 15 and 30 days with MC of 15%, 20% and 25% at 10°, 25°,30° and 35°C. To achieve hermetic conditions, plates were sealed and covered with aluminum foil and incubated for 30 days to create controlled climatic conditions. Finally, the AFs detection in rice sample was carried out using validated HPLC method with fluorescence detection.

Results: The results showed that the concentration of AFB_1 was increased with an increase in MC.Maximum growth of AFs was observed at 30°C with 25% of moisture content (MC) after 15 and 30 days incubation period. Whereas, minimum growth of AFs was recorded at 30°C in 15% MC after 15 days incubation period, proven through number of studies that temperature range from 25 -30°C supports growth of AFs with 18% -25% MC. Extreme growth were found on the plates incubated for 30 days as compared to 15 days incubation, as maximum period were consumed for AFs to grow well. While, at 10°C least concentration of Afs was recorded with 15% and 25% MC, which is evident to the studies disclose that AFs doesn't produce

at low temperature even in humid environment. Whereas, inhermetic storage, maximum concentration of toxins were discovered at 25° with 25% MC and minimum growth at 30°C and 15% MC. The results shows that the increased of temperature, the AFs concentration decreases with minimum MC (Hassane et al., 2017). Aerobic conditions are highly favorable for the growth of *Aspergillus* species and production of AFs in food commodities. This study also reveals that the optimum temperature range and increase in MC provide favorable conditions for fungus to grow well and produce more toxins. These conditions indicated that the production of AFs highly influences by climatic conditions.

Conclusion: The climatic conditions such as temperature and moisture content conditions are highly influence on the production of Afs content. Temperature that provides favorable conditions with moisture content is 30°C as proved through this research as well. Apart from temperatures, humidity also supports growth of AFs during drying period. Toxins were observed in sealed plates extra preventive measures like ultra-hermetic storage after solar drying without any expensive cost is better alternative. Properly sealed materials can reduce the threat of AFs growth in storage process of food commodities. According to the food safety point of view, the result can be used to develop numerous food safely strategies, like drying and storage of rice. The lower temperature (< 20 oC) and MC (< 10%) are best situations to control deterioration and AFs production in rice. In addition, the hermetic bag is the best option to control moulds growth and AFB_1 production in rice.

Keywords: Aflatoxins, Climatic conditions, Fungus, Food security, Storage.

ACKNOWLEDGMENT

The authors are very thankful to the Food and Feed Safety Laboratory / FMRRC of Pakistan Council of Scientific and Industrial Research (PCSIR) for providing well-equipped laboratory to conduct research work.

REFERENCES

1. Alshannaq, Ahmad F., and Jae-Hyuk Yu. “A liquid chromatographic method for rapid and sensitive analysis of aflatoxins in laboratory fungal cultures. “*Toxins* 12.2 (2020): 93 during peanut roasting. Food Res Intern.2017; 97:178–183.
2. Hassane, A. M. A., et al. "Influence of different moisture contents and temperature on growth and production of aflatoxin B1 by a toxigenic Aspergillus flavus isolate in wheat flour." *J. Ecol. Heal. Environ* 5 (2017): 77-83.
3. Martins, Ligia M., et al. "Kinetics of aflatoxin degradation during peanut roasting." *Food Research International* 97 (2017): 178-183.
4. Norlia, Mahror, et al. "Modelling the effect of temperature and water activity on the growth rate of Aspergillus flavus and aflatoxin production in peanut meal extract agar." *International journal of food microbiology* 335 (2020): 108836.
5. Odjo, Sylvanus, et al. "Hermetic storage technologies reduce maize pest damage in smallholder farming systems in Mexico." *Journal of Stored Products Research* 88 (2020): 101664.
6. Pekmez, Hatice. "Cereal storage techniques: A review." *J. Agric. Sci. Technol.* B 6 (2016): 67-71.
7. Tahir, Nasir Ishaque, et al. "Nature of aflatoxins: Their extraction, analysis, and control." *Journal of Food Safety* 38.6 (2018): e12561.
8. Yogendrarajah, Pratheeba, et al. "Mycotoxin production and predictive modelling kinetics on the growth of Aspergillus flavus and Aspergillus parasiticus isolates in whole black peppercorns (Piper nigrum L)." *International journal of food microbiology* 228 (2016): 44-57.