STUDIES ON THE GROWTH INHIBITION OF AFLATOXIGENIC FUNGI

Aspergillus flavus IN GROUNDNUT

M. Sangeetha*,
PG Department of Microbiology, A.V.C College (Autonomous), Mannampandal, Tamil Nadu, India.

Abstract

Aspergillus flavus is a fungi and it grows by producing thread like branching filaments known as hyphae. Aflatoxin producing members of Aspergillus are common and wide spread in nature. It can be pathogenic on several plants and animal including human being. It can infect seeds of corn, peanuts, cotton, and nuts trees. The native habitat of Aspergillus is in soil, decaying vegetation, hay and grains. The toxin can also be found in the milk of animals that are fed contaminated feed. To minimize the risk of aflatoxin exposure, close tripartite cooperation among the trade, the public and the government is essential. The prime responsibility to ensure the wholesomeness of the foods lies with the trade. They are advised to adapt the Good manufacturing Practice (GMP) and integrate it with HACCP based safety programme.

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1. Introduction

Aflatoxin belongs to a group of fungal toxins known as mycotoxins, and is widespread in agricultural products and food. Aflatoxin is associated with both acute and chronic toxicity in animals and humans including acute liver damage, liver cirrhosis, and liver cancers. We evaluated the aflatoxin surveillance from 1998 to 2000 in this report. Throughout this period, a total of 526 food samples, under the three food groups namely ground nuts and ground nut products, vegetable oil and fat, cereal and cereal products, were taken for aflatoxin analysis and the findings were compared against the statutory limits stipulated in the Harmful substances in Food Regulations of Public Health and Municipal Services Ordinance (Peterson et al., 2001). The contaminated foods reported to be associated with aflatoxin in Asian region include maize, peanuts, rice and other oil products. Accumulation of aflatoxin is dependent upon weather conditions. Before harvest, the risk for the development of aflatoxin is greatest during major droughts. When soil moisture is below normal and temperature are high, the number of Aspergillus sp. and spores in the air increases (Jelinek et al., 1989). Human exposure to aflatoxin is principally through ingestion of contaminated foods. Aflatoxin can cause both acute and chronic toxicity in animals. Effects such acute liver damage, liver cirrhosis, induction of tumours and teratogenic and other genetic effects are well documented. In modern days, acute toxicity of aflatoxin to humans has been encountered only rarely. Symptoms may include fever, vomiting and jaundice. Acute liver damage can occur which may be fatal in severe cases. Long term intake of aflatoxin can be associated with hepatic cancer. Animal studies have showed that hepatocellular
liver tumors may develop in animals like rats, hamsters and monkeys after prolonged oral administration. The younger generation is the one to find the solutions to future threats to the continents’ food security and livelihoods of its inhabitants. Improvements in the health of ecosystems will go a long way in protecting and promoting biodiversity. Biological control, if carefully developed and implemented, is the greenest approach to saving farmlands, waterways, savannas, and forests from the ravages of pests.

2. Materials and Methods

Spoiled ground nut collected from local market in Mayiladuthurai. Aspergillus flavus isolated from ground nut which had previously appeared to be different in their production of aflatoxin were selected for these studies. At the beginning of the experiments, transfers were made from each of the isolates on to Wort agar in petridishes (100 by 15mm). After the cultures had grown and sporulated, they were kept at 5 °C. All spores for inoculations in the experiments were obtained from these two plates. A. flavus isolates were grown on Wort agar, since chemical extraction from mycelia and spores on and above the agar surface is simpler and quicker than extraction from a liquid medium. In growth experiments, Wort broth medium was used because the subsequent drying and weighing of the mycelia and spores is accomplished more easily from a liquid medium. Therefore, aflatoxin production versus growth in the two types of media can be compared qualitatively but not quantitatively. Inoculations were made by transferring spores into either 80 ml of Wort agar in 500 ml conical flasks or into 50 ml of Wort broth in 300 ml flasks. After inoculation, all flasks were kept in the laboratory at 22 °C for 18 to 20 hr to insure spore germination before they were placed in the various temperature boxes. A test experiment was also conducted to determine whether the inoculation method would carry over any aflatoxin and whether the germinating spores would produce any aflatoxin during the initial 20 hrs period. Then replicate flasks for the A. flavus isolates were inoculated and then extracted after 20 hrs. No aflatoxin was recovered from any of the flasks. Temperatures of 2 to 29 ±0.5 °C were maintained in Biological Oxygen Demand Incubators temperature of 35 to 52 ±1.0 °C was maintained in incubators. The amount of growth was determined by pooling contents of three replicate flasks, washing off the nutrient solution and weighing the dried combined mycelia mass.

2.1. Aflatoxin production at various temperatures

In an initial short term experiment, inoculated flasks were incubated for 5 days, and aflatoxin was extracted within 1 to 2 hrs after harvest. A second, long term experiment (growth period of 3, 6 and 12 weeks) was done at temperatures (2 °C, 7 °C, 13 °C and 41°C) at which aflatoxin was not produced by the two A. flavus cultures in the initial short term experiments; each was done in four replicates. Control flasks were also inoculated and kept in the laboratory at 22 °C. In all instances, isolates in control flasks grew rapidly, sporulated and produced large amounts of aflatoxin.

3. Results and Discussion

Maximal total production of aflatoxin was obtained when cultures were grown at a temperature of 24 °C. Growth at this temperature also resulted in maximal production of each of the aflatoxin by A. flavus isolates. It was found that the ratio of production of aflatoxin to may be greatly changed when A. flavus is grown at certain temperatures. Maximal growth was not correlated with maximal aflatoxin production of aflatoxin. Production of aflatoxin was nil for isolates at temperatures of 2 °C, 7 °C, 13 °C, 41 °C, 46 °C and 52 °C. The intensity of the yellow color of the CHC13 extracts correlated with the concentration of aflatoxin.

Mateles and Adye (1965) observed a similar correlation between aflatoxin production and the yellowing of mycelium and substrate. The biochemical significance of this correlation between color and production of aflatoxin by A. flavus has not been investigated. Mahoney and Moleynex (2000) investigated the potential for factors naturally present in the crop to confer resistance to Aspergillus colonization and growth
or suppress aflatoxin biosynthesis. No aflatoxin B1 is not produced at 36 °C.

They also found that aflatoxin G1 is not produced at 18 °C; production starts between 18 and 24 °C and is at maximum at 30 °C. When those experiments which showed no aflatoxin formation after 5 days were extended to periods of up to 12 weeks, aflatoxin production occurred at one of the temperature formerly negative. These results were of additional interest because they indicated that aflatoxin may be remetabolized by one of the isolates (M122) after 6 weeks. A similar phenomenon was noted in A. parasiticus, which coincides with the attainment of maximal growth and onset of autolysis.

4. Reference


