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STUDIES ON AFLATOXIN PRODUCTION BY SEED-BORNE ASPERGILLI OF BAJRA (*PENNISETUM TYPHOIDES*)

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Eighteen isolates of *Aspergillus* were separately grown on modified Czapek's medium at 28 (±1) °C and the metabolites were tested for the presence of aflatoxins by thin-layer chromatography. Artificially infected seeds stored under controlled conditions of temperature and humidity and naturally infected seeds stored at laboratory, farmer's and trader's godowns were also tested for aflatoxins. Bajra seeds of local variety were found to be infected by a strain of *Aspergillus flavus* which produced aflatoxin B_1 in the synthetic medium as well as in artificially infested seeds. Naturally infected seeds by *A. flavus* stored at farmer's godown, after 10 months storage, were found to be contaminated with aflatoxin B_1.

INTRODUCTION

Spenseley in 1963 discovered that certain strains of *Aspergillus flavus* invading groundnut, produce a group of highly toxic substances known, collectively, as aflatoxins. Subsequent researches have shown that aflatoxins are produced on a wide variety of agricultural products (Hesseltine *et al.*, 1968; Barnes & Young, 1969; Shank *et al.*, 1972; Shotwell *et al.*, 1974) as well as by *Aspergillus* spp. other than *A. flavus* (Scott *et al.*, 1967; Wilson *et al.*, 1968). During storage of food-grains, Aspergilli produce aflatoxins which find their way in the food chain and cause serious ailments like liver damage. Since several species of *Aspergillus* were detected during storage of bajra these fungi were screened for aflatoxin production.

MATERIALS AND METHODS

Eighteen strains of *Aspergillus* were isolated during storage of bajra seeds collected from Agra, Anand, Bijapur, Guntur, Hissar, Hyderabad, Jodhpur, Kovilpatti, Sholapur and Varanasi. For detecting aflatoxins, culture filtrates of 18 isolates of *Aspergillus* grown on modified czapek's medium (Maggon *et al.*, 1969), bajra seeds artificially infested by different Aspergilli and the naturally infected seeds stored in Agra at farmer's house, trader's godown and Botany laboratory, Agra College were tested. Thin layer chromatography (Pons *et al.*, 1968) was followed.

Surface sterilized seeds, weighing 12g were rolled on 2 weeks old sporulating cultures of Aspergilli. The infested seeds were stored for 21 days at 28(±1) °C

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in small jute bags kept in dessicators wherein 88% and 55% relative humidities, respectively, were maintained following Clayton (1942). Finely ground dry material, weighing 10g was used for aflatoxin extraction. The spots were confirmed with those of known aflatoxins.

RESULTS

Only one strain of *Aspergillus flavus* (conidial) isolated from the seeds of local variety collected at Agra, was found to be toxigenic. Ten other strains of *A. flavus*, two of *A. nidulans* and one each of *A. chevalieri*, *A. niger*, *A. quadrilineatus*, *A. stellatus*, *A. versicolor* did not produce aflatoxins both at 88% and 55% R.H.

In the first screening of fungal metabolites produced in synthetic medium for aflatoxins, *A. flavus* (Agra) gave bluish flourescent spots comparable to standard aflatoxin B_1. Considering that the fungus may be able to produce aflatoxin in a natural substrate, all the isolates of *Aspergillus* were tested for toxin production on bajra seeds. Aflatoxins were not produced at 55% R.H. Only the toxigenic strain of *A. flavus* produced aflatoxin B_1 on the seeds stored at 28(±1)°C in 88% R.H. and the bluish flourescent spots were detected.

Aflatoxins were not detected in the seeds stored in 3 different locations, after 4 and 8 months storage. However, B_1 aflatoxin was detected in the seeds stored at farmers' house after 10 months storage.

DISCUSSION

Wilson *et al.* (1968) screened 13 species of *Aspergillus*, 3 species of *Fusarium*, 9 species of *Penicillium*, *Rhizopus nigricans*, *Pithomyces chartarum*, *Stachybotrys atra* and *Thielevia terricola* and found that only *Aspergillus flavus* and *A. parasiticus* gave positive results. These species have also been reported as aflatoxin producers by Boller and Schroeder (1966 & 1974a). Kulik and Holaday (1966) have reported 5 species of *Aspergillus* viz., *A. flavus*, *A niger*, *A parasiticus*, *A. ruber* and *A. wentii* to be toxigenic. Aflatoxin production by *Aspergillus ostianus* was reported by Scott *et al.* (1967). In the present investigations 18 isolates from bajra seeds, including 11 of *Aspergillus flavus* and 2 of *A. nidulans*, were tested for aflatoxin production on both synthetic medium and on infected seeds. Only one conidial strain of *A. flavus* was found to be toxigenic. The frequency of the toxigenic strain, as against bajra, has been found to be more on other crops. Mehan and Chohan found that 16 out of 21 isolates of *A. flavus* isolated from maize, cotton and wheat were toxigenic whereas 72 out of 240 isolates from groundnut samples, collected from 7 states of India, produced aflatoxin B_1 (Subramanyam & Rao, 1974). The toxigenic strain of *Aspergillus flavus* from bajra seed produced aflatoxin B_1 at 28(±1)°C. This temperature has earlier been reported to be suitable for aflatoxin production by Dierner and Davis (1966) and Mehan and Chohan (1974). Aflatoxin was earlier reported to be produced at 100% R. H. (Boller & Schroeder, 1974a, b) but in the present study aflatoxin B_1 was produced at 88% R.H. also.

In seeds invaded by a large number of fungi, including *Aspergillus flavus*, during storage at farmer's house, trader's godown & laboratory, aflatoxin B_1 was detected only in one out of 12 samples tested and that too only after 10 months storage under farmer's conditions. There was no aflatoxin production after 4 or 8 months storage. This scarcity of aflatoxin production may possibly be due to lack of required conditions of humidity and temperature, which should be around 80-90% and 25-30°C, respectively. During the storage of bajra the first four months at Agra (November-February) though humid to some extent (63-84% R.H.) are cool enough to retard aflatoxin metabolism. During the next four months (March-June) though the temperature range (22-28°C) is suitable but humidity is considerably low. In the months of July August the R.H. increases sufficiently due to rains and temperature remaining around 25-30°C, the aflatoxin production, therefore, starts after 10 months. Non-production of aflatoxin at trader's godown and laboratory may be due to interaction of toxigenic strain of *Aspergillus flavus* with other fungal species. Inhibition of aflatoxin production by *A. parasiticus* in rice has been reported in the presence of *A. chevalieri* (Boller & Schroeder, 1973) and *A. candidus* (Boller & Schroeder, 1974b). In the present investigations *Aspergillus candidus* was isolated from seeds stored at all the 3 locations but the percentage of infected seeds at farmer's place was much less. *Aspergillus chevalieri* was detected in the laboratory only. It may, therefore, be presumed that fungal species like *A. Chevalieri* and *A. candidus* may be influencing, adversely, the aflatoxin production in bajra under natural conditions of storage.

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