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RESEARCH ARTICLE

Incidence of toxigenic isolates of *Aspergillus flavus* in the aerospora of main fruit and vegetable market at Agra

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ABSTRACT

Concentration of air borne fungal spores in main fruit and vegetable market of Agra during June 2013 to May 2014 was studied by culture plate exposure method. This study revealed the presence of 42 fungal species in a period of one year. Aspergillus flavus was found to be most dominant and frequent mould in the aerospora of fruit and vegetable market. Maximum number of isolates of Aspergillus flavus (20) were recorded in the month of September 2013, while the minimum number of isolates (08) were noted in the month of June 2013 and January 2014. Out of a total of 153 isolates, 95 were found to elaborate variable quantity of aflatoxin B1 and G1, thereby indicating aflatxigenic nature of 62.09% isolates. The aflatoxin B1 was produced in the range of 200-2250 ppt, while the aflatoxin G1 was elaborated in the range of 150 – 500 ppb. No isolate was found to produce aflatoxin B2 and G2. This study showed that more than 60% isolates of Aspergillus flavus have potential to elaborate aflatoxin B1, which is potent hepatocrarcinogenc. If the spores of toxigenic isolate are inhaled regularly, then they may cause bronchial disorders including lung cancer.

Keywords: *Aeromycoflora, Aspergillus flavus, Aflatoxin, vegetable market*

INTRODUCTION

Many air borne organisms, as well as particles of biological origin passively float in the atmosphere. Moulds are frequent contaminants of fruit and vegetables. Air borne fungi are also considered as a key factor and as indicator of the level of air pollution. Many investigations on the aeromycoflora have been carried out in order to correlate with different type of allergic disorders in humans and vegetable diseases in India. (Sreeramula and Seshavataran 1962; Sreeramula and Ramalingam 1964 & 65; Tilak and Kulkarni 1972 & 80; Tiwari 1999). Occurrence of aeromycoflora in vegetable and fruit market have also been studied by Sharma and Bhattacharjee (2001), Medhi and Sharma (2010) and Kakde and Kakde (2012). The study of fungal aerospora of market may have some implications on the health of people working in the market, sellers and customers. Fungal propagules in ambient air are regularly and continuously inhaled by human beings. These fungal spores present in the atmosphere of vegetable and fruit market may be causative agents of respiratory diseases in humans and infections to various perishable commodities. Some genera of airborne fungal spores such as *Alternaria, Aspergillus, Fusarium* and *Cladosporium* are found throughout the world. (Pepeljnjak and Segvic Klaric 2003).

Besides, allergic disorders, these fungi are known to produce highly toxic metabolites called mycotoxins. Among them, aflatoxins are most important. The aflatoxins are a type of mycotoxin produced by *Aspergillus flavus*, which is a common fungus found in soil, air and decaying plant residues. The spores of *Aspergillus flavus* are found in abundance in air of fruit and vegetable market Desai and Ghosh (1989) reported aflatoxin related occupational hazards among rice mill workers of Gujrat. Later, Ghosh *et al.* (1997) noted air borne aflatoxin in the dust and aerosphere of grain producing industries in India. Singh and Prasad (2001) studied population of toxigenic isolates of *Aspergillus flavus* in dust and aerosphere of grain godowns in Bihar. Mould concentration in the ambient air of the busiest markets exhibit immense importance in order to study the dissemination of post harvest pathogens

found in the air and in evolving the forcasting system for post harvest and storage diseases of fresh fruits and vegetables (Sumbali and Bodyal 1991). In view of the above facts, the present investigation was undertaken to study aeromycoflora of main fruit and vegetable market at Sikandra, Agra and to find out incidence of toxigenic isolates of *Aspergillus flavus* and their potential to produce various aflatoxins.

MATERIALS AND METHODS

In the present study, aeromycoflora was student from ten sites of wholesale fruit and vegetable market of Sikandra, Agra. This study was carried out during 2013-2014 (June 2013 to May 2014) and observation were taken in 1st week of every month for the presence of fungi in the air. The culture plate exposure method (Kakde *et al*. 2001) was adopted for isolation of the aeromycoflora. At each site, 10 petriplates containing PDA medium were exposed for five minutes. The exposed plates were incubated at 28 ± 1ºC for 7 days in B.O.D. incubator. After incubation period, the plates were examined for the presence of *Aspergillus flavus* colonies. The isolates of *Aspergillus flavus* were identified, purified by subculturing and then maintained in PDA slants for further study.

The isolates of *Aspergillus flavus* were grown in SMKY liquid medium (Diener and Davis 1966) for production of aflatoxins. For this purpose, 50 ml of liquid medium was taken in 250 ml Erlenmeyer flask and sterilized at 15 lb pressure for 20 minutes in autoclave.

After cooling, the medium was inoculated with 0.5 ml spore suspension of the 7 days old culture of isolate of *Aspergillus flavus*. Then, the flasks were incubated at 28 ± 1ºC for 10 days in B.O.D. incubator. Each set was run in triplicate. After incubation period, the contents of the flask were filtered through Whatman No. 1 filter paper and culture filtrate was collected in sterilized flask.

For analysis of aflatoxins, ten ml of culture filtrate was extracted twice with two 10 ml proportions of chlorform in a separating funnel. Then this extract was passed through anhydrous sodium sulphate to remove moisture. This chloroform extract was evaporated to near dryness and the residue was dissolved in 1 ml chloroform and stored in dry small injection vial and later used for qualitative and quantitative estimation of aflatoxins by TLC following method outlined by Thomas et al. (1975) and Nabney and Nabbitt (1965).

RESULTS AND DISCUSSION

In the present study, *Aspergillus flavus* was found to be most dominant mould in the aeromycoflora of fruit and vegetable market of Agra. It was present in the observations of all the 12 months, thereby showing 100% frequency and 7.6 density. In all 153 isolates of *Aspergillus flavus* were isolated. The maximum number (20) of isolates of this fungus were recorded in the month of September 2013 while the minimum number (08) was noted in June 2013 and January 2014. Season wise, the maximum number of isolates of *A. flavus* were noted in monsoon season while the minimum number (30) of isolates could be noted in summer season. This can be attributed to the fact that most congenial conditions of environment occur in monsoon season for the proliferation of moulds. During this season high humidity coupled with suitable temperature favour growth of fungi. Further, more vegetable garbage is available during monsoon season in vegetable market (Sabji Mandi). During winter season, temperature is low and limits the proliferation of fungi, while summer season has high temperature and low humidity, which adversely affect the growth of fungi (Sullia and Khan 1980).

Out of 153 isolates of *Aspergillus flavus* obtained in this study, 95 produced aflatoxin B1 and G1 in varying quantity, thereby indicating 62.09% aflatoxingenic nature of the isolates. No isolate produced aflatoxin B2 and G2. In general, aflatoxin B1 was produced in the range of 200 – 2250 ppb while aflatoxin G1 could be elaborated in the range of 150 – 500 ppb only. It is needless to say that aflatoxin B1 is highly toxic among all the mycotoxins known till date (Krogh 1987). It is interesting to note that all the toxigenic isolates produced aflatoxin B1 and G1. The former was produced in more quantity then the latter. The isolates of *Aspergillus flavus* obtained in the month of September 2013 produced maximum quantity of aflatoxin B1

and G1 while the minimum quantity of these toxins was produced by isolates obtained in the month of January 2014. In Israel, Brout and Joffe (1965) reported that 71.2% isolates of *Aspergillus flavus* collected from groundnut soils and Kernels were toxigenic. A summary of the investigations conducted in six countries viz., U.K., U.S.A., Holland, South Africa, Israel and India revealed that 60% isolates of *Aspergillus flavus* were toxigenic (Diener and Davis 1969). Chauhan *et al.* (2011) also noted that 60.6% isolates of *Aspergillus favlus* obtained from root tubers of *Chlorophytum borivilianum* were aflatoxigenic, thereby supporting findings of present study.

Presence of 60% aflatoxigenic isolates of *Aspergillus flavus* in aeromycoflora of fruit and vegetable market (Sabji Mandi) is definitely harmful to human population, because such places are visited daily by large number of traders and consumers. If spores of such a toxigenic fungus are constantly inhaled, then human beings are sure to suffer from bronchial disorders including cancer of lungs as aflatoxin B1 is a potent carcinogen.

Table 1: Aflatoxin producing potential of isolates of *Aspergillus flavus* obtained in aeromycoflora of fruit and vegetable market of Agra.

S. No.	Month	Total No. of isolates screened	No. of aflatoxigenic isolates	% of aflatoxigenic isolates	Range of aflatoxins (in ppB)			
					B1	B2	G1	G2
1	June 2013	12	08	66.66	300-1750	–	240-400	–
2	July 2013	08	05	62.650	250-1200	–	150-250	–
3	Aug. 2013	15	10	66.66	350-2000	–	250-500	–
4	Sep. 2013	10	11	55.00	500-2250	–	300-500	–
5	Oct. 2013	16	09	56.25	250-1700	–	250-400	–
6	Nov. 2013	15	10	66.66	250-1500	–	150-350	–
7	Dec. 2013	10	06	60.00	250-1250	–	150-300	–
8	Jan. 2014	08	05	62.50	200-1200	–	100-250	–
9	Feb. 2014	12	07	58.33	200-1250	–	150-250	–
10	Mar. 2014	15	10	66.66	250-1500	–	150-450	–
11	Apr. 2014	12	08	66.66	200-1250	–	200-300	–
12	May 2014	10	06	60.00	200-1200	–	200-400	–
		153	**95**	**62.09**	**200-2250**	**–**	**150-500**	**–**

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