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ORIGINAL ARTICLE

Incidence of Fusarium Species in Aerospora of Fruit and Vegetable Market at Agra and Their Mycotoxigenic Potential

Ajay Garg, Ratika Singh and Surendra Singh
Botany Department, Agra College, Agra
Email: drajaygarg009@gmail.com

ABSTRACT

During monthly isolation of aeromycoflora of wholesale fruit and vegetable market of Agra, conducted from June 2014 to May 2015, 4 speices of Fusarium viz. F. gramineraum, F. moniliforme, F. solani and F. oxysporum were recorded. All species except Fusarium oxysporum occurred throughout the year, thereby showing 100% frequency. However, F. oxysporum showed 80.33% frequency. Thus, it is clear that species of Fusarium are quite prevalent in aeromycoflora of fruit and vegetable market. Out of 68 isolates of these species, 41 isolates were found to be mycotoxigenic in nature as they produced zearalenone, trichothecene and fumonisin, thereby indicating toxigenic nature of 68.28% isolates. Mycotoxin zearalenone was produced by all the four species but Trichothecene and fumonisin were produced by Fusarium graminearum, F. moniliforme and F. oxysporum. The remaining species Fusarium solani could produce only zearalenone in the range of 200-350 ppb. Further maximum zearalenone was produced by F. graminearum is the range of 450 - 950 ppb and the minimum quantity of this toxin was produced by Fusarium solani. Likewise, maximum trichothecene was produced by F. graminearum in the range of 300 - 450 ppb. However, maximum fumonisin in the range of 450 - 850 ppb was produced by Fusarium moniliforme, occurrence of mycotoxigenic moulds in aeromycoflora is deterimental to human beings as their spores are constantly inhaled and may cause lung disorders.

Key words: *Aeromycoflora; Fusarium, Fusarial mycotoxins.*

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INTRODUCTION

Fruit and vegetable markets are known to contain several species of fungi in the aerospora as well as on the surface of fruits and vegetables out of these, various pathogenic fungi constitute a dominant component of air, which eventually settle on the surface of fruits and vegetables. They are major spoiling agents responsible for post harvest fruit spoilage, leading to significant losses (Salunkhe and Desai, 1984). It has been estimated that about 20 - 25% of the harvested fruits are decayed by pathogens during post harvest handling even in developed countries (Droby, 2006). In developing countries, post harvest and transportation facilities. Over 30% of fruit and vegetable produce is wasted during harvest, grading packing, transport, marketing and storage (Surendranathan, 2005). Thus, there occurs a considerable gap in food production and net availability to consumers. There is further widening of this gap due to fungal spoilage in terms of quantity and quality. Fruits contain high levels of sugars and nutrients and their low pH value make them vulnerable to fungal decay (Singh and Sharma, 2007).

Besides spoiling fruits and vegetables, the fungi are potentially pathogenic and toxigenic. The toxins produced by fungi are called mycotoxins, which are hidden poisons in food

stuffs. Some potent fungal toxins like aflatoxins, ochratoxins and patulin have been detected in fruits during storage (Drusch and Ragab, 2003). Most of these mycotoxins are neurotoxic and carcinogenic to humans. Some other air borne pathogenic fungi like *Alternaria* and *Fusarium* also produce mycotoxins. In present investigation, attempt has been made to record *Fusarium* species in aerospora of wholesale fruit and vegetable market at Agra and to study their mycotoxigenic potential in relation to production of fusarial toxins viz., Zearalenone, T-2 toxin, and Fumonisin.

MATERIAL AND METHODS

In the present investigation, aeromycoflora was studied from 10 sites of wholesale fruit and vegetable market situated at Sikandra, Agra. This study was carried out during 2014-2015 (June 2014 to May 2015) by collecting air samples on Czapek Dox Agar (CDA) medium contained in petriplates (Supplemented with streptomycin sulphate @ 0.06 g/L). Samples were collected in 1st week of every month for the duration of one year. At each site 10 petriplates were exposed for five minutes and then incubated at 28 ± 1ºC for 7 days in B.O.D. incubator. After, incubation period, the plates were examined for the presence of fungi and species of *Fusarium* were isolated for further study.

SCREENING OF ISOLATES OF FUSARIUM SPECIES FOR MYCOTOXIGENIC POTENTIAL

In all 68 isolates of four species of *Fusarium* viz. *Fusarium graminearium* (16), *F. moniliforme* (20), *F. solani* (14) and *F. oxysporum* (18) were screened for their capacity to produced fusarial mycotoxins i.e., zearalenone trichothecin and fumonisins in culture medium. The different mycotoxins were detected qualitatively as well as quantitatively by thin layer chromatography using different solvents as outlined by Kammiura *et al*. (1981) and Rottinghaus *et al*. (1992). For this purpose, each isolate of *Fusarium* species was inoculated on sterilized moist maize medium contained in 250 ml Erlenmeyer flask. Three replicates of each isolate of *Fusarium* were prepared and kept in B.O.D. incubator at 25 ± 1ºC for 2 weeks. The cultures were harvested and dried at 55ºC for 12 hours. After cooling, the content of each flask was ground with the help of mechanical blender. Then chemical extraction of fusarial toxins was done with the help of ethyl acetate by blending and fillering with whatman No. 1 filter paper. The residue was then extracted with 250 ml of methanol: water (3:1). The extract was evaporated to near dryness in water bath and the final residue was dissolved in 1 ml methanol and reserved for thin layer chromatogrphy.

For thin layer chromatography, clean uniform glass plates (20 x 20 cm) were used. Then 36 g of silica gel-G was mixed with 72 ml of distilled water to form a slurry and it was used immediately for uniform coating of six glass plates at a thickness of 0.25 mm with the help of an applicator. The plates were dried at room temperature and then activated in an oven at 120ºC for an hour. Finally, sample concentrate of methanol-water was spotted on TLC plates along with known concentration of pure standards of fusarial toxins. Then these plates were developed in solvent comprising of chloroform: methanol: acetic acid (6: 3: 1). The solvent was allowed to run upwards for a distance of 15 cm and thereafter, the plates were air dried and examined under UV light in chromato view cabinet. The fusarial toxins were visualized with p-anisaldehyde spray and identified by comparing the Rf value of known standards. The quantitative estimation of these mycotoxins was done by "dilution to extinction procedure" as suggested by Jones (1972).

RESULTS AND DISCUSSION

In the present investigation 4 species of *Fusarium* viz., *Fusarium ghraminearum, F. moniliforme, F. oxysporum* and *Fusarium solani* were encountered in the aerospora of whole sale fruit and vegetable market at Sikandra, Agra. It in interesting to note that 3 species of *Fusarium* (*F. graminearum, F. moniliforme* and *F. solani*) showed 100% frequency as they occurred in the observations of all the twelve months and were considered as co-dominant moulds. It is worth while to point out that *Aspergillus flavus*

and *Alternaria alternata* were dominant moulds in this study. However, *Fusarium oxysporum* could be recorded from July 2014 to April 2015 and was absent in the observations of May and June 2015. Thus, it showed frequency of 80.33% (Table 1). It is quite clear that species of *Fusarium* are quite common in aeromycoflora. It will not be out of place to mention here that isolates of *Fusarium* species are potentially capable of producing about a dozen mycotoxins, of which zeaalenone, Trichothecene (T-2 toxin) and fumonisins are very important in relation to human health hazards.

Table 1: Incidence of *Fusarium* species during 01.06.14 to 30.5.15 in aerospora of wholesale fruit and vegetable market at Sikandra, Agra (Average no. of colonies per plate based on 100 plates)

S. No.	Name of Fungi	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
1.	*Fusarium graminearum*	4	8	8	8	8	6	5	6	8	8	6	4
2.	*Fusarium moniliforme*	6	6	4	4	4	6	5	4	6	8	4	3
3.	*Fusarium oxysporum*	3	4	6	6	6	4	4	4	6	6	-	-
4.	*Fusarium solani*	4	6	6	5	5	5	4	5	6	4	4	2

Table 2: Production of fusarial toxins (Zearalenone, Trichothecene and Fuminosin) by isolates of Fusarium species

S. No.	Name of species	No. of isolates screened	No. of toxic isolates	% of toxigenic isolates	Range of Fusarial toxins (in ppb)		
					Zearalenone	Trichothecene	Fumonisin
1.	*Fusarium graminearum*	16	10	62.50	450-950	300-450	200-350
2.	*Fusarium moniliforme*	20	13	60.50	300-400	150-300	450-850
3.	*Fusarium oxysporum*	14	06	42.85	200-350	-	-
4.	*Fusarium solani*	18	12	66.66	350-650	250-400	350-700
	Total	68	41	60.28			

Perusal of Table - 2 indicates that out of 68 isolates of *Fusarium* sp. 41 isolates were found to be toxigenic, thereby suggesting toxigenic nature of 68.28% isolates. Out of 16 isolates of *Fusarium graminearum*, only 10 isolates produced zearalenone in the range of 450 to 950 ppb, trichothecenes in the range of 300 - 350 ppb and fumonisin in the range of 200 - 350 ppb and was adjudged as highly toxic among species of *Fusarium* screened. The next important species was *Fusarium oxysporum* as its 12 isolates, out of 18, produced zearalenone, trichothecene and Fumonisin in the range of 350 - 650 ppb, 250 - 400 ppb and 350 - 700 ppb respectively. Interestingly, isolates of *Fusarium moniliforme* produced maximum quantity of humonisin (450 - 850 ppb) as compared to other species screened but it produced lower amounts of zearalenone (300 - 400 ppb) and trichothecene (150 - 300 ppb). The minimum toxigenic potential was shown by isolates of *Fusarium solani*. Only 42.85% isolates of this fungus were found to be toxigenic and these isolates could elaborate only zearalenone in the range of 200 - 350 ppb. The other two mycotoxins viz. trichothecene and fumonisin could not be elaborated by any strain of *Fusarium solani*.

Practically all reports about aeromycoflora of different places indicate that *Fusarium* species are quite frequent along with species of *Aspergillus, Penicillium* and *Alternaria* (Ghosh *et al*., 1997; Desai and Ghosh, 1989; Ahire and Sangale 2012; Ahmed *et al*., 2013; Vermani *et al*., 2014). The fungi of aerospora have been considered important in relation to allergic disorders of human beings. However, the most frequent and dominant moulds of aerospora are mycotoxigenic also. Some work regarding aflatoxin production by air borne spores of *Aspergillus flavus* has been carried out at some places, but no information

regarding other toxigenic moulds of aerospora are on record. This is the first report of production of fusarial toxins by *Fusarium* species obtained from aerospora of fruit and vegetable market at Agra.

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REFERENCES

1. Ahmed, J., Hossain, K.S. and Bashar, M.A. (2013). Aeromycoflora of the Dhaka University Campus. Bangladesh. J. Bot. 42 (2); 273-278.
2. Ahire, Y.R. and Sangale M.K. (2012). Survey of aeromycoflora present in vegetable and fruit market. Elixir Appl. Botany 52; 11381 - 11383.
3. Desai, M.S. and Ghosh, S.K. (1989). Aflatoxin related occupational hazards among rice mill workers. J. Toxicol. Toxin, Rev. 8; 81-88.
4. Droby, S. (2006). Improving quality and safety of fresh fruits and vegetables after harvest by the use of biocontrol agents and natural materials. Acta Horticul 709; 45 - 51.
5. Drusch, S. and Ragab, W. (2003). Mycotoxins in fruits, fruit jucies and dried fruits. J. Food Prot. 66(8), 1514-1527.
6. Ghosh, S.K., Desai, M.R. Pandeya, G.L., Venkaiah K. (1997). Airborne aflatoxin in the grain producing industries in India. Am. Ind. Hyg. Assoc. J. (5); 583 - 586.
7. Jones, B.D. (1972). Methods of aflatoxin analysis. Tropical products Institute, London pp. 9 - 70.
8. Kamimura, H., Nishijima, M., Yasuda, K., Satio, K, Ibe, A., Nagayma, T., Oshiyama, H. and Naoi, Y. (1981). Simultaneous detection of Fusarial toxins. J. Assoc. off Anal. Chem. 64; 1067 - 1073.
9. Rottinghaus, G.E., Coatney, C.E. and Minor, H.C. (1992). A rapid sensitive thin layer chromatography procedure for the detection of fumonisin B1 and B2. J. Vet. Diagn. Invest. 4; 326 - 329.
10. Salunkhe, D.K. and Desai, B.B. (1984). Post harvest biotechnology of fruits. Boca Rata, F.L. CRC Press, 168.
11. Singh, D. and Sharma, R.R. (2007). Post harvest diseases of fruit and vegetables and their management. In sustainable pest management Eds. D. Prasad, Daya Publishing House, New Delhi.
12. Surendranathan K.K. (2005). Post harvest biotechnology of fruits with special reference to banana - perspective and scope. Indian J. Biotech 4; 39 - 46.
13. Vermani, M., Bedi, N. and Hussain, M.S. (2014). Prevalence of culturable airborne fungi in fruit markets of Delhi and Noida, India, Int. Res. J. Environment Sci. 3(7); 1-6.