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ORIGINAL ARTICLE

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

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ABSTRACT

In all 5 species of Alternaria viz., A. alternata, A. brassicae, A. raphani, A. solani and A. triticina were isolated in aeromycoflora of wholesale vegetable and fruit market at Agra during 2014. Out of these Alternaria alternata was most frequent and abundant mould in all the 12 months of 2014. However, A. brassicae and A. raphani were more frequent in monsoon and winter season but remained absent in summer months. Interestingly Alternaria solani was present in all the months except June, but Alternaria triticina was recorded in the months of December, January, February, March, April and May and remained absent in monsoon months. These species of Alternaria were screened for their ability to produce alternariol (AOH), alternariol methyl ether (AME) and altenuene (ALT), the important mycotoxins of this mould. The isolates of Alternaria triticina, A. alternata and A. solani produced all the three mycotoxins, while isolates of A. brassicae and A. raphani could produce AOH and AME only. The AOH was produced in maximum quantity (200 – 850 ppb) followed by AME (100 – 450 ppb) by all the species of Alternaria screened. However, Altenuene was produced in lowest quantity (100 – 250 ppb) by Alternaria alternata, A. solani and A. triticina. The presence of toxigenic isolates in occupational environment is definitely alarming as the spores of these forms may be constantly inhaled by human beings and may colonize some vital parts like nasal tract, respiratory tract and lungs, thereby causing harmful effects.

Key words: *Alternaria, Fruit and Vegetable market, Aerospora, Mycotoxins.*

INTRODUCTION

The genus *Alternaria* is well represented by many species found ubiquitously and includes both plant pathogenic and saprophytic species that may damage crops in the field or cause post harvest disorders in agricultural products. Atleast 20% of agricultural spoilage is caused by *Alternaria* species. Christensen *et al.* (1968) reported that 53 of the 60 isolates of *Alternaira* from food and feed were found to be lethal when corn-rice cultures were fed to weanling rats. Later, Meronuck *et al.* (1972) showed that 20 of 23 isolates of *Alternaria alternata* were lethal to rats. Dawood (1982) reported that *Alternaria alternata* was the most frequent fungus in freshly harvested cereal grains and *Aspergillus flavus* and *Alternaria alternata* were most dominant moulds in stored seeds.

Scott (2001) reported that *Alternaria alternata* is a frequently occurring species on various crops causing leaf spot disease and it produces a number of toxic metabolites (Mycotoxins) including alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), altertoxin (ATX) and tenuazoic acid (TeA). Natural occurrence of some of these toxins have been reported in various fruits including tomatoes, olives, mandarines melans, peppers, apple and rasp berries. They have also been found in processed fruit products such as apple juice, other fruit beverages and tomato products. *Alternaria alternata* is probably next to *Aspergillus flavus* in producing mycotoxins and occurs on cereals, oil seeds, pulses, fruits and vegetables in field as well as storage. Liu *et al.* (1992) reported etiological role of *Alternaria alternata* in human oesophageal cancer. They suggested that AME and AOH could combine with DNA, isolated from human fatal oesophageal epithelium, activate the oncogenes and produce proliferation of human fatal oesophagal epithelium in *In vitro* conditions. Further, squamous cell carcinoma of the fatal oesophagus could be induced by AOH.

As such, the occurrence of *Alternaria* toxins in food stuffs such as grains, nuts, fruits and vegetables is becoming an increasing environmental concern. It is needless to say that *Alternaria* spores are one of the most common and potent indoor and outdoor air borne allergins. Additionally, *Alternaria* sensitization has been determined to be one of the most important factors in the onset of child hood asthma. Many human health disorders can be caused by *Alternaria* species which grow on skin and mucous membranes particularly on the eye balls, cornea, oral and sinus cavities, nails and within respiratory tract (Vennewald *et al*., 1999). The density of *Alternaria* increases during harvesting and threshing of crops and causes allergic disorders in farmers.

In the present study, *Alternaria* species have been isolated from atmosphere of main fruit and vegetable market (Sabji Mandi) of Agra at monthly interval for one year and their mycotoxin producing potential has been determined so as to know their seasonal occurrence and mycotoxigenic nature.

MATERIALS AND METHODS

In the present investigation, aeromycoflora was isolated from ten sites of wholesale vegetable and fruit market at Sikandra, Agra. This study was carried out during 2013-2014 and observations were taken every month for presence of fungi in the atmosphere. The culture plate exposure method was adopted for trapping the mycoflora. At each site 10 petriplates containing semisolid medium were exposed for five minutes every month. The exposed plates were incubated in B.O.D. incubator at 28 ± 1ºC for seven days. After incubation period, the petriplates were examined for presence of *Alternaria* species. These species were purified, sub-cultured and then identified with the help of keys given by Barnett and Hunter (1972) and Smith (1969).

The different isolates of *Alternaria* species were screened for their mycotoxigenic potential following Bilgrami *et al.* (1994). For this purpose, the isolates were grown in 250 ml flasks on a sterilized medium containing 50g of whole rice grains moistened with 75 ml of a nutrient solution comprising of 2% yeast extract in commercial Czapek's Dox-broth. The flasks were inoculated with 0.5 ml of spore suspension made from 7 days old culture of *Alternaria* species. The inoculated flasks were incubated in stationary culture for 21 days at 25ºC in B.O.D. incubator. The experiment was carried out in triplicate for each isolate of *Alternaria* species. After incubation period, the alternariols were extracted by homogenizing the entire culture in a waring blender for one minute with acetone : water (70 : 30 v/v) and allowing the slurry to stand for 15 minutes. Then the slurry was filtered under suction through glass wool and was concentrated to 25% of its original volume with an air stream and placed in the cold at 18ºC for 24 hrs, so as to obtain a precipitate. The precipitate was filtered and re-dissolved in dioxane and placed at room temperature until crystals are formed. The crystals were dissolved in 1 ml methanol and analysed for the presence of alternariols by thin layer chromatography following Seitz *et al.* (1975).

RESULTS AND DISCUSSION

The perusal of Table 1 indicates that only 5 species of *Alternaria* could be isolated in aeromycoflora of fruit and vegetable market (Sabji Mandi) at Agra during the year 2014. These include *Alternaria alternata, A. triticina, A. brassicae, A. solani* and *A. raphani.* Out of these, *Alternaria alternata* was quite dominant and frequent as it was only species, which was present in the observation of all 12 months. The next important was *Alternaria solani* as it was recorded in all the months except June 2014. Interestingly, *Alternaria brassicae* and *A. raphani* were more frequent in rainy and winter season but remained absent in summer months. It may be due to the fact that these species attack members of Brassicaceae, which are grown in monsoon and winter seasons. In a survey of small grains in the United States, species of *Alternaria* were found in 184 samples, out of a total of 230 samples screened for the association of moulds (Bruce *et al*., 1984). Stinson *et al.* (1980) recorded strains of *Alternaria* from apples, tomatoes, oranges, lemons and blue berries.

Alternaria spores are one of the most common and potent indoor and outdoor air borne allergins (Vinnewald *et al*., 1999). Scott (2001) reported that *Alternaria alternata* is a frequently occurring species in field and storage. It produces a number of mycotoxins, of which alternariol (AOH), alternariol methyl ether (AME) and altenuene (ALT) are more important.

In the present investigation, *Alternaria* species isolated in aeromycoflora of Sabji Mandi were screened for their ability to produce above mentioned mycotoxins in culture medium. This study revealed that more than 60% isolates of *Alternaria alternata, A. triticina, A. brassicae* and *A. solani* were toxigenic. However only 58.33% isolates of *A. raphani* were toxigenic. Interestingly isolates of 3 commonly found species of *Alternaria* viz. *A. alternata, A. triticina* and *A. soloni* produced all the 3 types of alternarioles (AOH, AME and ALT) in variable quantity (Table 2). The isolates remaining two species i.e. *Alternaria brassicae* and *A. raphani* could elaborate only alternatiol and alternariol methyl ether in low quantity and they could not produce altenuene. The capacity of a isolate to produce a particular mycotoxin depends mainly on its genetic set up. However, the amount of toxin produced is affected by cultural and environmental conditions.

Table 1: Occurrence of *Alternaria* species in aerospora of fruit and vegetable market (Sabji Mandi) Agra during 2014

S. No.	Name of Species	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1.	***Alternaria alternata***	8	8	10	10	6	6	8	8	10	8	8	7
2.	***A. triticina***	6	6	7	8	4	3	–	–	–	–	–	2
3.	***A. brassicae***	8	7	7	5	–	–	–	2	5	5	6	6
4.	***A. solani***	7	8	6	3	3	–	4	5	5	6	4	5
5.	***A. rephani***	5	4	3	2	–	–	–	2	2	2	3	4

Table 2: Production of Alternariols by isolates of *Alternaria* species in culture

S. No.	Name of Species	No. of isolates screened	No. of toxic isolates	% of toxigenic isolates	Conc. of alternariols (in ppb)		
					AOH	AME	ALT
1.	***Alternaria alternata***	46	30	65.21	250-850	150-450	150-250
2.	***A. triticina***	20	15	75.00	200-750	150-400	100-250
3.	***A. brassicae***	20	12	60.00	250-750	150-300	–
4.	***A. solani***	32	20	65.50	200-800	100-400	150-250
5.	***A. rephani***	12	7	58.33	200-600	100-200	–

AOH = Alternariol
AME = Alternariol methyl ether
ALT = Altenuene

This study also revealed that isolates of *Alternaria alternata* are main producer of alternariols as they could produce alternariol, in the range of 250 – 850 ppb. Alternariol methyl ether was produced in the range of 150 – 450 ppb but altenuene could be produced only in the range of 150 – 250 ppb. Almost similar range was shown by isolates of *A. triticina* and *A. soloni* for these toxins. Muller (1992) studied toxin producing ability of species of *Alternaria* and noted that *Alternaria alternata* and *A. tenuissima* produced the highest amount of AOH, AME and ALT. He also noted that out of 87 strains, 77 produced

alternariol and alternariol methyl ether, while 50 produced tenuazonic acid and only 18 strains produced altenuene. Kavyanjali (2011) isolated 83 isolates of *Alternaria alternata* from pre harvest, post harvest and stored wheat grains samples. She reported that 66.26% isolates were toxigenic as they could produce alternariol, alternariol methyl ether and altenuene in variable quantity, thereby supporting findings of present study.

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