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# INCIDENCE OF MYCOTOXIN PRODUCERS ON SPICES FROM ANDHRA PRADESH

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Ten different spices were investigated for mould infestation and mycotoxins incidence by collecting samples from 21 districts of Andhra Pradesh State. High counts of moulds were observed on seeds of, mustard, black pepper, ginger, caraway and chilli. The fungal flora consisted of 122 species belonging to 35 genera. Among these *Aspergillus* (20 species), *Penicillum* (25 species), *Fusarium* (17 speices), *Curvulari* (7 species) and *Alternaria* (6 species) along with Mucoraeae were dominant. Ginger followed by black pepper and mustard supported maximum number of toxigenic fung, while cloves supported least number of toxigenic moulds.

**Key Words :** Mycoflora, Mycotoxin, Spices, Aflatoxin, Incidence.

Natural contamination of agricultural produce with mycotoxins is fairly high in developing countries like India (Aktar and Khan, 1987). The mycoflora and mycotoxigenic fungi of a variety of food commodities were reported by Bilgrami (1984) . Keeping in view, the same present investigation was followed.

## MATERIALS AND METHODS

Ten different spices (Table 1) were analysed for the degree of mould infestation and for their mycotoxigenic potential. The media used for the isolation of fungi were PDA, Asthana and Hawkers medium A and Dichloron Rose Bengal medium. The samples were analysed by blotter technique (Anoymous, 1985) and dilution plate method (Waksman, 1922).The fungi isolated and identified were screened for mycotoxin production by employing standard methods (Scott *et al.*, 1970; Udagawa *et al.*, 1978, Kamimura Rao *et. al.*, 1985). Percentage of frequency of individual fungus was calculated by the following formula:

$$\text{Percentage of frequency} = \frac{\text{Number of observations in which a species appeared}}{\text{Total number of observations}} \times 100$$

## RESULTS AND DISCUSSION

Overall 122 fungal species belonging to 35 genera contributed to the mycobita. The species belong to Acomycotina, Deuteromycotina and Zygomycotina.

Species variation was highest in *Penicillium* (25) followed by *Aspergillus* (20), *Fusarium* (17) and *Curvularia* (7). Together with fungi of Mucoraceae, these moulds dominated the fungal spectrum of the samples.

The mycoflora of mustard seeds was dominated by *Aspergillus flavus, A, fumigatus* and species of *Penicillium*. Nustard seeds supported the growth of 58 fungal species representing 22 genera (Table 2).

Seeds of black pepper "the king of spices" hosted a wide range of fungal species (Table 2). *Aspergillus fumigatus* and *A. japonicus dominated* the fungal spectrum. This was followed by *A. ochraceous* and species of *Penicillium*, *P. citrinum* and *P. oxalicum* dominated the *Penicillium spectrum. Dendrophopsis* sp., *Humicola grisea* and *Gonyrichum macrocladum* were sporadic in their incidence. Interestingly, actinomycetes were also detected by blotter technique with a significant percentage of incidence (10 to 50%).

The aromatic cremocarps of caraway supported the growth of 55 fungal species representing 26 genera (Table 2). Thirteen species of *Aspergillus*, 5 of *Fusarium* and 6 of *Penicillium* were isolated from caraway cremocarps.

The dry fruits of red pepper (Chilli) favoured the growth of 46 fungal species representing 19 genera (Table 2). Species of *Aspergillus* dominated the fungal spectrum followed by species of *Fusarium* and *Penicillium.*

A comparatively low incidence of fungi in unopened dried flower buds of cloves may be attributed to the presence of eugenol. Ubiquitous fungi like

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Table 1: List of spices examined and their infestation

| Name of species | Number of samples collected | *Number of samples +ve for presence of fungi | **Number of samples with toxigenic fungi | % of myco-toxigenicity |
|---|---|---|---|---|
| Black pepper (*Piper bigrum* L.) | 45 | 41 | 4 | 48.07 |
| Caraway (*Carum carvi* L.) | 43 | 41 | 2 | 31.18 |
| Chilli (*Capsicum annuum* L.) | 45 | 42 | 12 | 30.64 |
| Cloves (*Eugenia caryophyllata* L.) | 45 | 35 | - | 23.52 |
| Fenugreek (*Trigonella foenum graecum* L.) | 44 | 40 | 8 | 28.93 |
| Garlic (*Allium sativum* L.) | 42 | 38 | 12 | 31.97 |
| Garlic rhizome (*Zinziber officinale* Roxb). | 43 | 42 | 10 | 61.94 |
| Mustard (*Brassica nigra* Kich) | 45 | 42 | 11 | 35.80 |
| Onion bulb (*Allium cepa* L.) | 44 | 39 | 7 | 35.00 |
| Turmeric Corm (*Curcuma longa* L.) | 45 | 38 | 9 | 35.84 |

* On the basis of blotter technique and dilution plate method
** Natural incidence of fungi.

*Aspergillus niger, A. fumigatus, Rhizopus stolonifer, P. citinum* and *P. oxalicum contrinuted* to the fungal biota of this spice, while the occurrence of other fungi was only limited (Table 2). Blotter technique was not of much use in the detection of fungi probably due to the antimicrobial principle present in the cloves.

Studies on the mycobiota of an important condiment like fenugreek seeds reveal the association of 50 fungal species representing 17 genera (Table 2). The incidence of *Aspergillus species* of *Penicillium, Curvularia, Fusarium and chaetomium* were the other contaminating fungi.

Rhizome of ginger supported extremely high number of species of *Fusarium and Penicillium. P. citrium, P. frequentans and P. purpurogenum mainly* contributed to the *Penicillium spectrum,* while to that of *Fusarium. F. graminearum., F. oxysporum* and *F. moniliforme. A. flavus and A, fumigatus* dominated the mycobiota spectrum. A total of 48 fungal species representing 18 genera were the components of mycobiota (Table 2) and was rich qualitatively.

The other important spice, garlic (bulb, cloves) were also a victim of storage mycobiota, *Aspergillus niger* and species of *Penicillium* dominated the mycobiotic spectrum. The incidence of other fungi was low (Table2, VIII). Spectrum of *Penicillium* was mainly dominated by *P, citrinum, P. oxalicum* and *P. purpurogenum. Sporotrichum* sp. was also traced on few samples.

Thirty five fungal species representing 16 genera were countered on onion bulbs (Table 2). *A, niger,* species of *Fusarium* and *Penicillium* were common. The incidence of pathogenic *Stemphylium vesicarium* and smut spores was also observed in many samples.

Only a limited number of fungi were associated with processed rhizomes of turmeric (Table 2). The species of *Aspergillus* which mainly contributed to the mycobiota were dominated by *A. flavus, A. fumigatus* and *A. ochraceus. A niger* and *A.japonicus* too were recorded infrequently.

In general the species of *Aspergillus and Penicillium* dominated the mycoflora of species. Most of the fungi isolated from these spices were reported to be mycotoxigenic and produced variety of mucotoxing. Critical perusal of Table 1 & 2 indicates that the mycotoxigenic potential of fungi associated with ginger was highest, followed by black pepper and black mustard, while association of such fungi was low in cloves and fenugreek. A total of 1638 fungal isolates were screened for mycotoxin production in the present study. 607 isolates (37.05%) were positive for one or other mycotixins. Aflatoxins were the most common mycotoxins produced by different isolates of *A. flavus* group. Trichothecenes were low in their incidence. Variation in the nature and incidence of mycoflora and mycotoxins associated with different spicy commodities may be attributed to various factors including suitability of

Table 2: Mould frequency and mycotoxins incidence on different spices

| Name of fungus | I | II | III | IV | V | VI | VII | VIII | IX | X | A | B | Name of mycotoxin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Alternaria altemata* (Fr.) Keiss | 6.6 | - | 11.1 | - | - | 19.9 | - | 6.6 | - | - | 36 | 9 | Altennence (8) Altemario L (1) |
| *Aspergillus flavus* Link | 42.1 | 24.1 | 44.4 | 57.7 | 27.4 | 39.9 | 37.7 | 88.8 | 32.2 | 31.0 | 222 | 110 | Aflatoxin B(85), ff B&B (25) |
| *A. fumigatus* Fresen | 37.7 | 42.1 | 39.9 | 48.8 | 36.4 | 46.7 | 35.5 | 66.4 | 33.5 | 35.5 | 212 | 104 | Gliotoxin (91), Sterigmatocystin (13) |
| *A. nidulans* (Gill arey) Bline & Johanides | 6.6 | 19.9 | 6.6 | 11.1 | 8.8 | 6.6 | 19.9 | 17.7 | 4.4 | 11.1 | 50 | 8 | Sterigmatocystin |
| *A. niveus* Blochwitz | 4.4 | - | - | - | - | - | - | - | - | - | 8 | 1 | citrinin |
| *A. ochraceus* Wilhem | 19.9 | 8.8 | 13.3 | 11.0 | 35.5 | 24.4 | 22.2 | 19.9 | 15.6 | 22. | 115 | 51 | |
| *A. parasiticus* Speare | 8.8 | - | 6.6 | 4.4 | - | 4.4 | - | - | - | 4.4 | 13 | | affatoxin B |
| *A. terreus* Thom. | 15.5 | 26.6 | 8.8 | 13.3 | - | 17.7 | - | 17.0 | - | - | 30 | 18 | Pantlin (11), terreic acid (7) |
| *A. ustus* Bain | 24.4 | 22.2 | 19.8 | 28.8 | 4.4 | 22.2 | 26.6 | 288 | 22.2 | 19.9 | 104 | 42 | Ustic acid |
| *A. versicolor* (Vuill) Tiraboshii | 17.7 | - | 33.3 | 11.1 | 22.2 | 24.4 | 15.5 | - | - | 6.6 | 66 | 2 | Citrinin |
| *Chaetomium globosum* Kunze ex Stens | 19.9 | 15.5 | 22.2 | 13.3 | 31.0 | 11.1 | 6.6 | 15.5 | - | 15.5 | 68 | 14 | Chactoglobosins |
| *Fusarium acuminatus* ellis & everhart | 2.2 | - | - | - | - | - | - | - | - | - | 4 | 2 | HT-2 toxin. |
| *F. avenaceum* (Fr.) Sacc. | - | - | 4.4 | - | - | - | - | - | - | - | 3 | 1 | diacetoxyscirpenol (DAS) |
| *F. culmorum* (W.G. Smith) sacc. | - | - | - | - | - | - | - | 4.4 | - | - | 2 | 1 | zearalenone (ze) |
| *F. dimerum* Penz. | - | - | - | - | - | - | - | 8.8 | - | - | 2 | - | |
| *F. equisetin* (Corda) Sacc. | - | 17.7 | - | - | - | - | - | - | 11.1 | - | 15 | 10 | deoxynivaleno L (3), DAS (2) NV (2), ze (4) |
| *F. fusariodes* (Frag & Cif) Booth | - | - | - | - | 2.2 | - | - | - | - | - | 1 | - | |
| *F. graminearum* Schwade | - | - | - | - | - | - | - | 11.1 | - | - | 3 | 1 | T-2 toxin (1), DAS (1) |
| *F. haterosporum* nees ex Ft. | - | - | - | - | - | 4.4 | 8.8 | - | 4.4 | - | 15 | 5 | Zearalenone |
| *F. merismoides* Corda | - | - | - | - | - | - | 2.2 | - | - | 2 | - | - | |
| *F. moniliforme* Sheldon | 8.8 | 4.4 | 8.8 | 11.1 | 2.2 | 6.6 | 11.1 | 8.8 | 17.7 | 6.6 | 66 | 34 | DAS(9), moniliformin (1), Ze (14). T-2 toxin (20) |
| *F. oxysporum* Shelecht | 4.4 | - | 11.1 | 8.8 | 2.2 | 8.8 | - | 11.1 | 8.8 | 8.8 | 52 | 27 | Fusarenon X(3), T-2(9), Ze (8), DAS(6) |
| *F. poae* (Peck) Woolenew | - | - | - | - | - | - | - | - | 4.4 | - | 6 | .2 | T-2 (1), HT-2 (1) DIN (1), NS (1) |
| *F. semitectum* Berk & Rav. | - | - | - | 6.6 | - | - | - | - | - | 4 | - | | |
| *F. salni* (mart) Sacc. | 2.2 | - | - | 4.4 | - | - | 17.7 | - | 8.8 | - | 24 | 6 | DAS (2) M neosolanio L (2), T-21 T-2(1) Ze (1), |
| *F. sporotrichoides* Sherb | - | 2.2 | - | - | 4.4 | - | 4.4 | - | - | - | 16 | 8 | Ze(4), HT-2(2), DOS(2) |
| *F. tabacinum* (beyma) w. Gams | - | 2.2 | - | - | - | - | - | - | - | - | 1 | - | |
| *F. tricinctum* (Corda) Sacc. | - | - | 2.2 | | - | - | - | - | - | - | 6 | 3 | T-291). DAS (1)HT-2(1) |
| *Memnoniella echinata* (Riv.)Galloway | 2.2 | 13.3 | 4.4 | 6.6 | - | - | - | 6.6 | 11.1 | - | 18 | - | - |
| *Myrothecium roridum* Tode ex Steudel | - | 2.2 | - | - | 4.4 | - | - | 19.9 | - | - | 15 | 3 | Roridin |
| *Penicillium atroventum* S. Smith | 2.2 | - | - | - | - | - | - | - | - | - | 1 | - | - |
| *P. aurantiogriseum* Dicreckx | 2. | - | - | 2.2 | - | 4.4 | - | - | - | - | 16 | 5 | Penitrem B |
| *P. canescens* Sopp | - | - | - | - | - | - | - | 2.2 | - | - | 4 | 1 | Ochratoxin A |
| *P. Chrysogenum* Thom | - | 2.2 | - | - | - | - | - | - | - | - | 2 | 1 | Cyclopiazonic acid (CPA) |
| *P. citrium* Thom | 13.4 | 8.8 | 11.1 | 13.4 | 8.8 | 17.7 | 32.5 | 19.09 | 34.5 | 8.8 | 84 | 3 | Citrinin (24), och A(8), citriovi-ridin (6) |
| *P. corylophilum* Dierckx | - | 2.2 | - | - | - | - | - | - | - | 2 | - | - | |
| *P. decumbens* Thom | - | 2.2 | - | 4.4 | - | - | - | 4.4 | - | - | 10 | 1 | citrinin |
| *P. expansum* Link ex Gray | 11.1 | - | - | - | - | - | - | - | - | - | 13 | 2 | CPA |
| *P. fellutanum* Biourage | - | - | - | - | - | - | 8.8 | - | - | 5 | - | - | |
| *P. frequentans* Westling | - | - | 8.8 | 4.4 | - | - | - | 8.8 | 17.7 | 4.4 | 31 | 10 | citrioviridine (6), och A (4) |
| *P. funiculosum* Thom4.4 | 4.4 | 2.2 | - | 6.6 | 2.2 | 6.6 | 2.2 | - | 6.6 | 25 | 3 | | Och. A 91). Och B (2) |
| *P. granulatum* Bain | 2.2 | - | - | - | - | - | - | - | - | - | - | 2 | - - - - |
| *P. griseofulvum* Dierckx- | 6.6 | - | - | - | 4.4 | - | - | - | - | 13 | 7 | 7 | CPA (4) Och, A(2) Patulin (1) |
| *P. islandicum* Sopp4.4 | 2.2 | - | - | - | - | - | - | 2.2 | - | 4 | 1 | | islandicin |
| *P. janthiellum* Biourage | - | - | - | - | - | 2.2 | - | - | - | 2 | - | - | |
| *P. jensenii* Zaleskii | 2.2 | - | - | - | - | - | - | - | - | - | 2 | - | |
| *P. oxalicum* Curie & Thom | 1 | 8.8 | 4.4 | 8.8 | 11.1 | 8.8 | 11.1 | - | - | 15.5 | - | 38 | 9aOch A94), Patulin (2), Roqefortine C(3) |
| *P. purpurogenum* stoll | - | - | - | - | - | | 13.4 | 8.8 | 11.1 | - | 21 | 6 | Secalonic acid D (4), Och A(2) |
| *P. restrictum* Gilman & Thom | - | - | 2.2 | - | - | 4.4 | 6.6 | 2.2 | - | 9 | - | - | |
| *P. rugulosum* Thom | 2.2 | - | - | - | - | - | - | - | - | - | 7 | 1 | Och A |
| *P. sponulosum* Thom | 2.2 | - | - | - | - | - | - | - | - | 2 | - | - | - - |
| *P. steckii* Zaleski | - | - | - | 2.2 | - | - | - | - | - | - | - | 2 | - |
| *P variable* Sopp | - | - | - | - | - | - | - | - | - | 6.6 | 3 | 1 | Och. A |
| *P verruculosum* varcyclopium (Westling Samson, Stolk & Hadlok | 4.4 | 2.2 | - | - | 2.2 | - | - | - | - | - | 10 | 4 | CPA (2), citrinin (1) Och A(1) |
| *P. verruculosum* varverrucosum Dierckx | - | - | - | - | - | - | - | - | - | - | 2.22 | - | |
| *P. viridicatum* Westling | - | 2.2 | - | - | - | - | - | - | - | - | 2 | 2 | Citrinin (1), patulin (1) |
| *Stachybotrys atra* Cords | 8.8 | 6.6 | 4.4 | 15.5 | - | 4.4 | - | 6.6 | - | - | 37 | 13 | Satratoxin D |
| *Trichoderma harzianum* Rifal | - | - | - | 11.1 | - | - | - | - | - | - | 9 | 2 | Gilotoxin |
| *T. koningii* Oudem | - | - | - | - | - | - | 8.8 | 4.4 | 15.5 | 4.4 | - | 15 | - |
| *T. viride* Pers ex Gray | 8.8 | 11.1 | 13.3 | 8.8 | - | 6.6 | 17.7 | 11.1 | 6.6 | 13.3 | 45 | 20 | Gliotoxin (14), Trichoderm- in (6) |
| *Trichothecium roseum* (Pers) Link ex Gray | 6.6 | - | - | - | - | - | - | - | - | - | 15 | 1 | Trichothecine |

**Other fungi Isolated :** *Absidia corymbifera, Acremonium terricola, Actinodictys sp. Alternaria dianthi, A. dianthicola, A. tenuissima, A. solani, Alternaria sps. Aspergillus chevaliero, A. flavipes, A. candidus, A. japonicus, A. ornatus, A. sclerotium, A. sulphureus, A. sydowii, Beltraniella humicola, Chaetomium brasiliensis, Cladosporium chlorocephalum, C. herbarum, C. oxysporum, Colletotrichum falcati, Curvularia clavata, C. geniculata, C. lunata, C. pallescens, C. penniseti, C. erogrostoides, C. seleganesis, Dendrophiopsis* sps., *Drechslera holodes, D. hawaiensis, D. rostrata, D. spicifer, Epicoccum purpurascens, Gonytrichum macrocladum, Humicola grisea, Mucor various, M. circinel loides, Nigrospora oryzae, Oedocephalum* sps. *Paecilomyces varioti, Phoma sorghina, Rhizoctonia salani, **Rhizopus stolonifer, *Scolecobasidium constrictum, Scopulariopsis brevicaulis, Spegazzinia tessarthra, Sporotrichum* sps., *Synephalastrum racemosum, Ulocloadium botrytis, Verticillium alboatrum, V. roseum*

*pre dominate fungi **fungi with % of frequency 5.0-15.0, I=Mustard, II=Black pepper, III=Caraway, IV=Chilli, V=Cloves, VI=Fenugreek, VII=Ginger, VIII=Garlic, IX=Onion, X=Turmeric

A=Total no. of strains screened; B=Total no. of mycotoxin producing strains.

substrate, climatic conditions, storage practices etc. Fluctuating temperature, high humidity especially during monsoon in this area are favourable for the growth of toxigenic fungi any mycotoxin elaboration.

As most of these mycotoxins have deleterious effects on man and animals (Betina, 1984), their incidence on spices should be matter of concern. Pungent mustard, aromatic cremocarps of caraway, rhizomes of ginger, corm of turmeric and seeds of black pepper are of relatively poor substrates for aflatoxin, gliotoxin citrinin and fusarial toxins risk (Ueno, 1983). The incidence of these mycotoxins at low concentration which is difficult to be detected makes the problem more critical. The potential occurrence of more than one mycotoxin and tendency of mycotoxins bind to the substratum in which they are produced, are some of the problems which need serious attention (Linderfelser *et al.*, 1974).

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