Indian J.Sci.Res.2(3) :71-74, 2011

# TOXIGENIC MOULDS ASSOCIATED WITH ROOT TUBERS OF SAFED MUSLI (*Chlorophytum borivilianum* Sant. F)

**YASHASVITA CHAUHAN[a1], R. K. SINGH[b], RATIKA SINGH[c] AND S. SINGH[d]**

Mycology Research Lab, Department of Botany, Agra College, Agra, U.P., India
[a]E-mail: yashasvita.tulip@gmail.com
[c&d]E-mail: dr.ssingh2010@yahoo.co.in

## ABSTRACT

**Divine herb *Chlorophytum borivilianum* (Safed Musli), family Liliaceae, is an aphrodisiac, antiageing health restorative and health promoting medicinal plant. The present study revealed that a total of 55 fungal species were recorded from dried and fresh samples of safed musli (root tubers).These fungi mainly include 10 species of *Aspergillus*, 3 species of *Penicillium*, 2 species of *Fusarium* and 1 species each of *Alternaria*, *Cladosporium*, *Curvularia*, *Drechslera*, *Helminthosporium*, *Mucor, Mycelium sterila*, *Rhizopus, Trichoderma, Botrytis, Candida, Cephalosporium, Cunnighmella, Paecilomyces, Trichothecium* and *Verticillium*. Among these, the most prevalent mould was *Aspergillus flavus*. In all 95 isolates of *Aspergillus flavus* were isolated and subjected to preliminary screening for aflatoxigenic nature. Out of these, 70 isolates showed aflatoxigenic nature in CAM ammonia test. The other dominant moulds were *Fusarium* and *Penicillium* which are known to elaborate mycotoxins. The association of toxigenic moulds with root tubers of safed musli needs a serious consideration in regard to human health as it is gaining wide spread acceptance as an alternative to Viagra**

**KEYWORDS**: Safed Musli, *Aspergillus flavus*, aflatoxin

Herbal drugs have been known for millennia and are now in great demand all over the world as a rich source of therapeutic agents for the cure of diseases and ailments. In India over 6000 plants are in use in traditional folk and herbal medicines representing about 75% of the medicinal needs of third world (Sharma et al., 2008).Safed musli (*Chlorophytum borivilianum* Sant. F), a miraculous herb of family liliaceae is distributed in certain pockets of Maharashtra, Baster forest (M.P.), Dang forest (Gujarat) and Arawali ranges of Rajasthan (Vartak 1959). It plays a paramount role in Indian herbal medicine as an aphrodisiac, antiageing health restorative and health promoter. It is also known as 'desi-viagra'and its medicinally important root tubers are rich source of 24 alkaloids, many vitamins, minerals, proteins, carbohydrates, saponins and steroids (Gondi et al., 2004)

Post harvest and storage spoilage of crude herbal drugs by moulds is one of the most important threats associated with production of herbal medicines in terms of quality deterioration and mycotoxin contamination (Roy, 2003; Dubey et al., 2008). Due to immense importance of safed musli, the present investigations were undertaken to evaluate predominant mycoflora and toxigenic strains of *Aspergillus flavus* associated with fresh and dried root tubers of this plant.

## MATERIALS AND METHODS

### Collection of Samples

Random samples were collected from farmers and traders of different places of Agra region in sterilized polyethene bags and were stored at $4^0$C in refrigerator till analysed.The analysis was made within 15 days for isolation of moulds.

### Analysis of Samples

### 1. Isolation and Identification of Moulds

The samples were screened for the association of moulds following standard methods viz; surface washing and serial dilution method and agar plate technique using PDA and CDA media (Waksman, 1927; Graves and Hesseltine, 1966).The plates were incubated at 28 $\pm 2^0$C for 7 days and observation was made on 7th day under microscope. The keys of Barnett (1960) and Gilman (1975) were used to identify the fungi. The identification of *Aspergilli* was made after Raper and Fennell (1965) and the isolates of fungi were maintained on PDA slants.

### 2. Primary Screening of Isolates of *Aspergillus flavus* for Aflatoxigenic Nature

The isolates of *Aspergillus flavus* were screened for their aflatoxigenic nature by CAM ammonia test as adopted by Kumar et al., (2006)

[1]**Corresponding author**

## RESULTS AND DISCUSSION

These studies revealed that a total of 55 fungal species were associated with dried and fresh samples of root tubers of safed musli. These fungi include 10 species of Aspergilli, 3 species each of *Alternaria, Cladosporium, Penicillium* and *Rhizopus*, 2 species each of *Acremonium, Bipolaris, Chaetomium, Curvularia, Fusarium, Helminthosporium, Mucor* and *Verticillium*, and one species each of *Botrytis, Botryotrichum, Cephalosporium, Colletotrichum*, *Cunnighmella, Drechslera, Monilia, Mycelia sterila, Myrothecium, Nigrospora, Paecilomyces, Pithomyces, Rhizoctonia, Stachybotrys, Trichoderma* and *Trichothecium*.Among these the most prevalent and dominant mould was *Aspergillus flavus* followed by *Fusarium oxysporum* and *Penicillium citrinum*. Roy (2003) also reported frequent occurrence of *Aspergillus, Fusarium* and *Penicillium* species on different crude herbal drugs. Abou et al.,(1999) and Gautam & Bhadauria (2008, 2010) noted occurrence of these fungi in Egyptian herbal drugs and in stored herbal fruit samples respectively, supporting findings of present investigations.

In all 95 isolates of *Aspergillus flavus* were isolated and subjected to preliminary screening for aflatoxigenic nature. Out of these, 70 isolates showed aflatoxigenic nature in CAM ammonia test thereby indicating aflatoxin producing ability of 72.63% isolates. Among these 20 isolates were found to be highly aflatoxigenic as they produced deep orange coloration and 26 showed moderate orange pigmentation, indicating, moderate aflatoxigenic nature. Further, light orange pigmentation was shown by 18 isolates and were treated as mild aflatoxigenic, while 6 isolates identified with very light orange pigmentation were regarded poorly aflatoxigenic. Borut and Joffe (1966) reported that 71.2% isolates of *Aspergillus flavus* collected from ground nut soils and kernels were aflatoxigenic thereby supporting our findings. Mishra (2008) also noted that 67.13% isolates of *A. flavus* isolated from wheat grains showed varying aflatoxigenic nature.

Since aflatoxins are known to have carcinogenic, teratogenic, hepatotoxic and mutagenic effects (WHO, 1979; IARC, 1993), their consumption via contaminated herbal products such as "Musli powder" may cause adverse health effects instead of curing diseases. So, association of toxigenic moulds with root tubers of safed musli needs a serious consideration as it is gaining widespread acceptance as an alternative to Viagra.

**Fig.: Varying degree of aflatoxigenicity of *Aspergillus flavus* isolates as exhibited by CAM ammonia test**

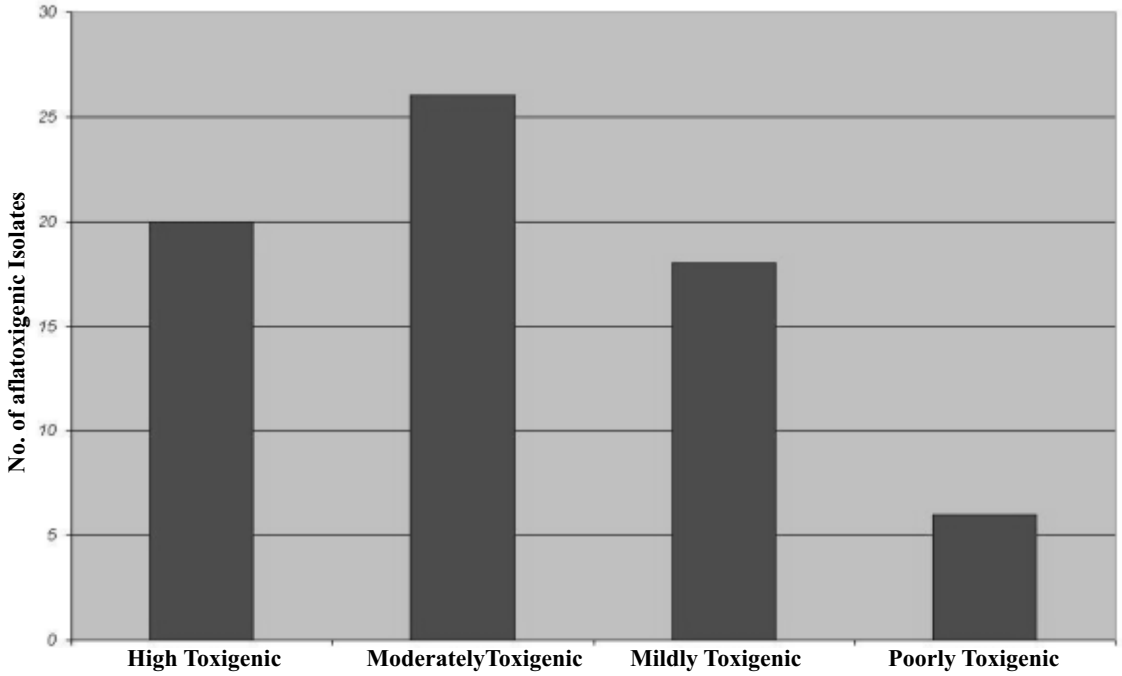

**Table: Distribution of fungi in dried and fresh samples of Safed Musli**

| S. No. | Name of Fungi | Dried | Fresh |
|---|---|---|---|
| 1. | *Acremonium alternatum* | + | - |
| 2. | *Acremonium vitis* | + | - |
| 3. | *Alternaria alternata* | + | + |
| 4. | *Alternaria solani* | + | - |
| 5. | *Alternaria triticina* | - | + |
| 6. | *Aspergillus candidus* | + | + |
| 7. | *Aspergillus clavatus* | + | + |
| 8. | *Aspergillus flavus* | + | + |
| 9. | *Aspergillus fumigatus* | + | - |
| 10. | *Aspergillus japonicus* | + | - |
| 11. | *Aspergillus nidulans* | + | + |
| 12. | *Aspergillus niger* | + | + |
| 13. | *Aspergillus parasiticus* | + | - |
| 14. | *Aspergillus sulphureus* | + | - |
| 15. | *Aspergillus terreus* | + | + |
| 16. | *Bipolaris rostrata* | - | + |
| 17. | *Bipolaris sarokiniana* | - | + |
| 18. | *Botrytis cinerea* | - | + |
| 19. | *Botryotrychum piluliferum* | - | + |
| 20. | *Candida albicans* | - | + |
| 21. | *Cephalosporium acremonium* | + | + |
| 22. | *Chaetomium globosum* | + | - |
| 23. | *Chaetomium spirale* | + | - |
| 24. | *Cladosporium cladosporoides* | - | + |
| 25. | *Cladosporium herbarum* | - | + |
| 26. | *Cladosporium sphaerospermum* | - | + |
| 27. | *Colletotrichum sp* | - | + |
| 28. | *Cunnighmella sp* | - | + |
| 29. | *Curvularia brachyspora* | + | - |
| 30. | *Curvularia lunata* | + | - |
| 31. | *Drechslera sp* | + | + |
| 32. | *Fusarium oxysporum* | + | + |
| 33. | *Fusarium roseum* | + | + |
| 34. | *Helminthosporium microsporum* | - | + |
| 35. | *Helminthosporium sativum* | - | + |
| 36. | *Monilia sitophila* | - | + |
| 37. | *Mucor circinalloides* | + | + |
| 38. | *Mucor haemalis* | + | + |
| 39. | *Mycelium sterila* | + | + |
| 40. | *Myrothecium roridum* | - | + |
| 41. | *Nigrospors oryzae* | - | + |
| 42. | *Paecilomyces terricola* | - | + |
| 43. | *Penicillium chrysogenum* | + | + |
| 44. | *Penicillium citrinum* | + | + |
| 45. | *Penicillium expansum* | + | + |
| 46. | *Pithomyces chartarum* | - | + |
| 47. | *Rhizoctonia solani* | - | + |
| 48. | *Rhizopus arrhizus* | + | + |
| 49. | *Rhizopus stolonifer* | + | + |
| 50. | *Rhizopus chinensis* | + | + |
| 51. | *Strachybotrys atra* | - | + |
| 52. | *Trichoderma viridae* | - | + |
| 53. | *Trichothecium roseum* | - | + |
| 54. | *Verticellium alboatrum* | - | + |
| 55. | *Verticellium theobromae* | - | + |

## ACKNOWLEDGEMENTS

Authors are thankful to Principal Agra College, Agra for providing us all kinds of facilities. We are also grateful to Dr. R. M. S. Sengar, associate professor of botany, for his persistent assistance and encouragements.

## REFERENCES

Abou A. A., Soliman K. M., El-Tantaway M. E., Ismail B. R. and Naguib K.,1999.Quantity estimation of some contaminants in commonly used medicinal plants in Egyptian market. Food Chemistry, **67**: 357-363.

Barnett H. L.,1960. Illustrated Genera of Imperfect Fungi, II ed., Burgess Publ. Co., Minneapolis.

Borut S. Y. and Zoffe A. Z.,1966. *Aspergillus flavus* and its toxicity on groundnuts in Israel. Israel J. Bot., **14**: 198-199.

Dubey N. K., Kumar A., Singh P. and Shukla R., 2008. Microbial contamination of raw materials: A major reason for the decline of India's share in the global herbal market. Current Sc., **95**: 717-718.

Gautam A. K. and Bhadauria, R., 2008. Occurrence of toxigenic moulds and mycotoxins in ayurvedic medicine Trifla churn. J. Indian Mycol. Pathol., **38**:664-666

Gautam A. K. and Bhadauria R., 2010. Fungal and mycotoxin contamination of some common stored herbal fruit samples. J. Indian Bot. Soc., **89**: 74-79.

Gilman J. C.,1975. A manual of soil fungi. Oxford and IBH Publ. Company, New Delhi.

Gondi M., Tyagi, S.K. and Srinivasan K., 2004. In Safed Musli. A white Gold. Agrobios (India) Publ. House, Jodhpur: 11-19.

Graves M. and Hesseltine C.W., 1966. Fungi in flour and refrigerated dough products. Mycopath et Mycol. Appl., **29**: 277-290.

IARC,1993. Some naturally occurring substances, food items and constituents, heterocyclic aromatic amines and mycotoxins. Monograph In: Agency for Res on Cancer, Lyon, France, **56**:244-295.

Kumar S., Shekhar, M., Khan A.A. and Chaudhari A., 2006. Detection of toxigenic and none toxigenic strains of *Aspergillus flavus*..Ind. Phytopath., **59**(3):379.

Mishra T., 2008. Studies on Potential of Biological Control of Aflatoxin Contamination in Wheat by Co-Existing Moulds. Ph. D. Thesis, Dr. B. R. A. Univ., Agra, U.P.

Raper K.B. and Fennell D.I., 1965. The genus *Aspergillus*. The Williams and Williams Co. Baltimore.

Roy. A. K., 2003. Mycological problems of crude herbal drugs: Overview and challenges. Indian Phytopath., **56**: 1-13.

Sharma A., Shankar C., Tyagi L. K. ,Singh M. and Rao C. V., 2008.Herbal medicines for market potential in India: An Overview. Academic J. Plant Sc., **12**: 26-36.

Vartak V.D., 1959. Some edible wild plants from the hilly region of the Poona district, Bombay State. J. Bombay Nat. Hist. Soc., **58**:8-25.

Waksman S.A.,1927. Principles of Soil Microbiology. Bailleire Tindall & Co. London: 19-27

WHO, 1979. Environmental health criteria. 11. Mycotoxins. World Health Organization, Geneva: 1-127.