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ORIGINAL ARTICLE

Association of Toxigenic Moulds with Dried Roots of *Rauvolfia Serpentina* in Agra

Ajay Garg
Botany Department, Agra College, Agra, U.P., India
Email: drajaygarg009@gmail.com

ABSTRACT

Dried roots of Rauvolfia serpentina have long been used as cure for schizophrenia, epilepsy, insanity and hypertension (high blood pressure). Present study revealed the presence of 29 fungal species associated with dried root samples of this plant, collected from traders of 5 tehsils of Agra districts. The dominant and most frequent moulds were Alternaria alternata, Aspergillus flavus, A. ochraceus, Fusarium oxysporum, F. moniliforme, Penicillium citrinum and P. patulum. Out of these, Aspergillus flavus was most frequent and abundant mould, which was found associated with all the samples of all the places. In all 69 isolates of Aspergillus flavus were recorded, out of which 48 were found to be aflatoxigenic, thereby showing 69.56% toxigenic nature of isolates. The presence of toxigenic isolates of moulds in crude herbal drugs is of serious concern in relation to human health as fungi not only deteriorate the active drug constituent but also make it toxic. This toxic drug may cause adverse health effects in patients instead of curing the disease.

Key words: *Toxigenic mouls, Rauvolfia serpentina, Aflatoxins*

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INTRODUCTION

Recently, considerable attention has been paid to utilize ecofriendly and biofriendly plant based products for the prevention and cure of different human diseases. Considering the adverse side effects of synthetic drugs, the western population is now looking forward for natural remedies, which are safe and effective. It is documented that about 80% of the world's population has now faith in traditional medicines particularly herbal drugs for their primary healthcare (Dubey, *et al.*, 2004).

In India, over 6000 plants are in use in traditional, folk and herbal medicines representing 75% of the medicinal needs of the third world countries (Rajsekharan, 2002). The dried roots of *Rauvolfia serpentina* have long been used by the people of India as a cure for epilepsy, high blood pressure, insanity, intestinal disorders and cardiac diseases. Its alkaloid reserpine was the first tranquiliser to be used for the treatment of schizophrenia and other forms of mental disorders. Being a hypotensive agent, reserpine is widely used today for curing hypertension (high blood pressure).

Post harvest and storage spoilage of crude herbal drugs by storage moulds is one of the most important threats with production of herbal medicines in terms of drug quality deterioration and mycotoxin contamination (Dubey, *et al.*, 2008). Due to immense importance of roots of *Rauvolfia serpentina*, the present investigation was undertaken to evaluate predominant mycoflora and toxigenic strains of *Aspergillus flavus* with dried roots of this plant, which are used as herbal drug.

MATERIALS AND METHODS

Random samples of dried roots of *Rauvolfia serpentina* were collected from traders of five sub urban areas viz. Khandoli, Kiraoli, Kheragarh, Shamsabad and Fatehabad of Agra district, in sterlized polythene bags and were stored at 4ºC in refrigerator till analysed. The analysis for association of moulds was made within 15 days of collection.

(I) Isolation and Identification of Moulds:

The samples were analysed for the association of moulds following standard methods viz., surface washing and serial dilution method and agar plate technique using PDA medium (Graves and Hesseltine, 1966). The plates were incubated at 28 ± 2°C for 7 days in B.O.D. incubator. After incubation period, the plates were studied for the presence of moulds by routine method under compound microscope. The different fungi were identified following Barnett (1960) and Gilman (1975). Finally, fungi were subcultured and different isolates of *Aspergillus flavus* were maintained on PDA slants.

(II) 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 outlined by Kumar, *et al.*, (2006). In this method, conidial mass of isolate of *Aspergillus flavus* was placed in the centre of petriplate containing coconut agar medium and incubation at 28 ± 1ºC for 7 days. Then about 5 ml of ammonia solution was poured on lid of petriplate and kept upside down for 30 minutes. Thereafter, plates were examined visually for the orange/brown pigmentation on the reverse side of plates. The intensity of pigmentation was expressed by the number of (+) sign and aflatoxigenic nature of the isolates was tentatively determined.

RESULTS AND DISCUSSION

Perusal of Table 1 indicates that a total of 29 fungi were found associated with dried root samples of *Rauvolfia serpentina* collected from trader of sub urban areas of Agra. These include 10 species of *Aspergillus (A. candidus, A. clavatus, A. flavus, A. fumigatus, A. japonicus, A. nidulans, A. niger, A. ochraceus, A. sulphureus and A. terreus*), 3 species each of *Fusarium (F. oxysporum, F. moniliforme, F. roseum*) and *Penicillium (P. citrinum, P. patulum, P. expansum*) and one spices each of *Acremonium, Alternaria, Botrytis, Cephalosporium, Chaetomium, Cladosporium, Curvularia, Mucor, Nigrospora, Paecilomyces, Rhizoctonia, Rhizopus and Trichothecium*.

Out of these moulds, Aspergillus flavus was most frequent and abundant mould associated with dried root samples of *Rauvolfia serpentine*. Other co-dominant moulds recorded in this study include *Alternaria alternata, Aspergillus japonicus, A. nidulans, A. ochraceus, Fusarium oxysporum, F. moniliforme, Penicillium citrinum, P. patulum* and *Penicillium expansum*. Interestingly, all these moulds are known to produce different mycotoxins which pose problem of health hazards in human beings (Krogh, 1987).

Further, association of moulds with crude herbal drugs may cause deterioration of active drug ingredient such as reserpine in this case and also make them contaminated with mycotoxins. In the present study, aflatoxigenic potential of isolates of *Aspergillus flavus* was evaluated by CAM ammonia test, which gives quick and reliable result about toxigenic nature of *Aspergillus flavus*. Perusal of Table 2 clearly suggests that in all 69.56% isolates of Aspergillus flavus were toxigenic. Mishra (2008) also noted that 67.13% isolates of *Aspergillus flavus* isolated from wheat grains showed varying degree of aflatoxigenic nature. Chauhan, *et al.*, (2011) recorded 95 isolates of *Aspergillus flavus* associated with root tubers of safed musli (*Chlorophytum borivlianum*), which is well know medicinal plant. In preliminary screening for aflatoxigenic nature, they noted that 70% isolates were potentially aflatoxigenic, which support findings of present investigation. Since aflatoxins and other toxins produced by species of *Alternaria, Fusarium* and *Penicillium* are potential

health hazards, their presence in crude herbal drugs should be taken seriously and efforts be made to minimize mould and mycotoxin contamination in crude herbal drugs.

Table 1: Moulds associated with dried root samples of *Rauvolfia serpentine*

S.No.	Name of Fungi	Khandoli		Kiraoli		Kheragarh		Fatehabad		Shamsabad	
		WT	AP	WT	AP	WT	AP	WT	AP	WT	AP
1.	*Acremonium vitis*	2	-	1	-	1	1	3	1	2	1
2.	*Alternaria alternata*	3	2	4	2	3	1	4	2	5	2
3.	*Aspergillus candidus*	-	-	1	-	2	1	2	1	2	-
4.	*A. clavatus*	3	1	2	1	3	2	3	-	2	-
5.	*A. flavus*	6	3	7	2	5	2	6	3	5	4
6.	*A. fumigatus*	4	2	3	1	3	-	4	2	3	1
7.	*A. japonicus*	3	1	2	2	2	1	3	2	2	2
8.	*A. nidulans*	4	2	1	1	3	2	2	1	3	1
9.	*A. niger*	2	1	3	2	2	1	-	-	2	2
10.	*A. ochraceus*	5	2	4	2	4	1	3	1	4	2
11.	*A. sulphureus*	-	-		-	1	-	1	-	-	-
12.	*A. terreus*	2	1	-	-	2	1	1	1	-	-
13.	*Botrytis cinerea*	-	-	1	-	-	1	-	-	1	-
14.	*Cephalosporium sp.*	1	-	-	-	1	-	-	1	-	-
15.	*Chaetomium spirale*	2	1	-	1	1	1	-	-	1	1
16.	*Cladosporium herbarum*	3	1	2	2	2	1	2	1	1	1
17.	*Curvolaria lunata*	2	1	3	1	2	-	2	-	-	-
18.	*Fusarium oxysporum*	4	2	5	2	3	1	4	2	5	2
19.	*F. moniliforme*	3	1	4	2	3	2	2	1	3	2
20.	*F. roseum*	2	1	2	-	2	1	2	1	3	2
21.	*Mucor haemalis*	2	1	-	-	2	1	2	2	-	-
22.	*Nigrospora oryzae*	1	-	1	-	-	-	2	-	-	-
23.	*Penicillium citrinum*	3	1	2	1	2	2	3	2	2	1
24.	*P. patulum*	4	2	3	1	3	2	3	1	3	1
25.	*P. expansum*	2	1	2	2	2	2	3	1	2	1
26.	*Paecilonyces terricota*	-	-	1	1	-	-	2	-	-	-
27.	*Rhizoctonia solani*	2	-	1	-	1	-	-	-	1	-
28.	*Rhizopus arrhizus*	3	2	2	2	2	1	2	-	1	1
29.	*Trichothecium roseum*	1	-	1	1	1	-	1	-	-	-
Total No. of Fungi		**24**	**21**	**26**	**18**	**25**	**20**	**25**	**19**	**20**	**15**

WT = washing technique; AP = Agar plate method

Table 2: Aflatoxigenic potential of isolates of *Aspergillus flavus* associated with dried root samples of *Rauvolfia serpentine*

S.No.	Place	Total isolates	Toxigenic isolates	% of aflatoxigenic isolates
1.	Khandoli	15	12	80.00
2.	Keraoli	12	09	75.00
3.	Kheragarh	13	08	61.50
4.	Fatehabad	16	10	62.50
5.	Shamsabad	13	09	69.20
Total		**69**	**48**	**69.56**

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