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RESEARCH ARTICLE

MYCOFLORA ASSOCIATED WITH THE "FRESH" AND "MARKET" ROOTS OF DRUG *Clerodendrum phlomoides* LINN. UNDER STORAGE AT DIFFERENT RELATIVE HUMIDITIES AND INCUBATION DAYS

Rashidi*¹ M. and Deokule² S. S

^{1,2}Department of Botany, University of Pune, Pune-411007- India *Corresponding author: Rashidi_129@yahoo.com

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ABSTRACT

Total 25 fungal species were isolated from fresh and market root samples of drug *Clerodendrum phlomoides* Linn. Out of these 18 fungi were associated with fresh samples and 13 fungi were isolated from market samples which 6 fungal species appeared in both samples. In the fresh samples, *F. solani* and in market samples *A. niger* recorded in high percentage incidence and they were the dominant fungi. Root samples were stored under different 30, 50, 75, 96 and 100 % relative humidity and different incubation days 15, 30, 45 and 60 days. Maximum growth and occurrence incidence of fungi were observed under high relative humidities 75, 96 and 100% RH and long storage periods 45 and 60 days.

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INTRODUCTION

Clerodendrum phlomoides Linn. is commonly called as "Urni" or "Urani". In Maharashtra called "Takali" belonging to family "Verbenaceae". In Mumbai the root is used as a bitter tonic and it's given in the convalescence of measles. Decoction of root which is slightly aromatic and astringent is used as a demulcent in gonorrhoea. This is also giving to children during convalescence from measles (Nadkarni, 1956 and Chopra *et al.*, 1958). Root and leaf extracts have been used for the treatment of rheumatism, asthma and other inflammatory diseases (Anonymous, 1992). Decoction of its root which is slightly aromatic and astringent is used as a demulcent in gonorrhoea board. Mycological problems that include association of mycoflora with crude herbals under storage, their role on biodeterioration and mycotoxins contamination have drawn worldwide attention for quality maintenance and therapeutic potentials of plant drugs. Relative humidity, temperature, light, oxygen, inocula and substrates are factors, which effect on development of storage fungi and mycotoxin contamination in stored herbal drugs. Among these, relative humidity and temperature have the most influence on the growth of fungi. There are not any report concentrating on the subject mycoflora associated with roots of *Clerodendrum phlomoides*, therefore, a survey was carried out to determine the percentage incidence of fungi associated with the roots of this drug in fresh and store condition and also effect of different relative humidity and incubation days on growth and percentage incidence of fungi were carried out.

MATERIAL AND METHODS

The fresh roots of drug *C. phlomoides* were collected in healthy, flowering and fruiting conditions from different

localities in Maharashtra, India. Market drug samples were also collected from various Shopkeepers and Kashthaushadhi. Samples were brought to the laboratory in polyethylene bags and were cut into small pieces and soaked for 2 minutes in 2 % sodium hypochlorite solution then thoroughly washed with sterilized distilled water. Ten pieces of roots placed in each Petri plate. Agar plate method (Potato Dextrose Agar, Water Agar, Czapek Dox Agar and Carnation leaf Agar) and Blotter test as recommended by International Seed Testing Association (1966) were done for isolation and identification of mycoflora associated with roots. For evaluation effect of incubation period and relative humidity on fungal growth, the root samples were stored in small muslin clothes and put under 30, 50, 75, 96 and 100 % RH in desiccators for 60 days in the room temperature. Internal 15, 30, 45 and 60 days, the root samples were taken out and thoroughly washed with distilled water and plated in Petri plates and they were incubated at 25^{0C} and after 3-4 days, developed colonies of fungi observed and the percentage incidence of mycoflora was recorded. Different fungi were identified up to genera and species level by using references, such as Raper and Thom (1949), Thom and Raper (1945), Barnet and Hunter (1972), Both (1971) Nelson *et al.* (1983) and Ellis (1971).

RESULTS

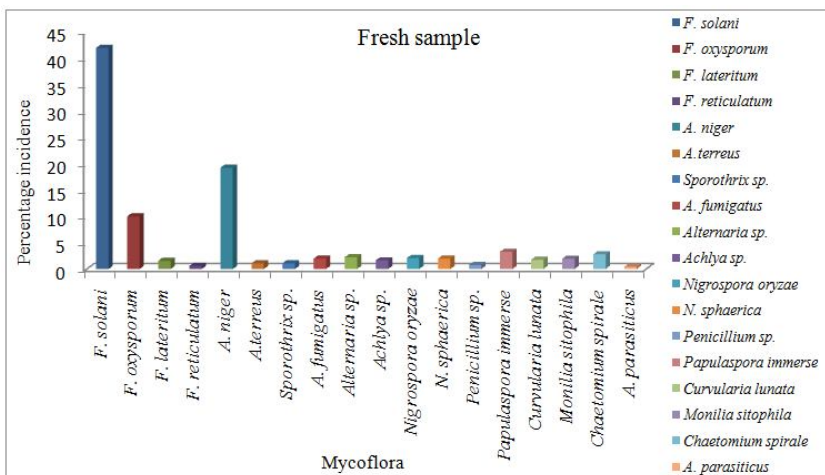
Mycoflora associated with fresh and market samples

The result of this study revealed that 18 fungi isolated from fresh samples of drug *C. phlomoides*, which include: *F. solani*, *F. oxysporum*, *F. lateritum*, *F. reticulatum*, *A. niger*, *A. terreus*, *Sporothrix* sp., *A. fumigatus*, *Alternaria* sp., *Achlya* sp., *Nigrospora oryzae*, *Nigrospora sphaericha*, *Penicillium* sp.,

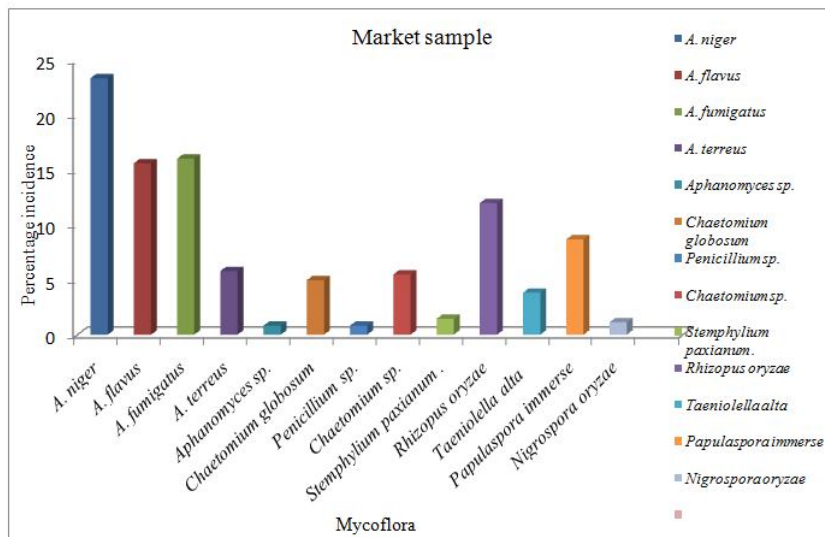
Papulaspora immerse, *Curvularia lunata*, *Monilia sitophila*, *Chaetomium spirale* and *A. parasiticus*. The highest percentage incidence was belong to *Fusarium* spp. that *F. solani* with 42.17% was dominant fungi, while *F. oxysporum*, *F. lateritum* and *F. reticulatum* observed 10.03, 1.5 and 0.6 percentage incidences respectively. *A. niger* with 19.3 % after *F. solani* had highest % incidence of fungi, *A. terreus*, *Sporothrix* sp., *A. fumigatus*, *Alternaria* sp., *Achlya* sp., *Nigrospora oryzae*, *Nigrospora sphaericha*, *Penicillium* sp., *Papulaspora immerse*, *Curvularia lunata*, *Monilia sitophila*, *Chaetomium spirale* and *A. parasiticus* observed in 1.07, 1.09, 1.97, 2.25, 1.64, 2.08, 1.98, 0.77, 3.25, 1.77, 1.96, 2.8 and 0.43%, respectively (Graph 1). Fungi were isolated from market samples of this drug, were *A. niger* (23.37%), *A. flavus* (15.61%), *A. fumigatus* (16.02%), *A. terreus* (5.79%), *Aphanomyces* sp. (0.82%), *Chaetomium globosum* (4.96%), *Penicillium* sp. (0.82%), *Chaetomium* sp. (5.48%), *Stemphylium paxianum* (1.44%), *Rhizopus oryzae* (11.99%), *Taeniolella alta* (3.82%), *Papulaspora immerse* (8.68%) and *Nigrospora oryzae* (1.13%), *A. niger* was found highest percentage incidence; *Penicillium* sp. and *Aphanomyces* sp. with same percentage incidence occurred in less percentage incidence (Graph 2).

Results of effect of relative humidity and incubation days on fungal growth

A perusal of results in Table 1 indicates that: roots of *C. phlomoides* stored under 30% relative humidity after 15 days of storage show 0.29 % total percentage incidence which increase to 3.48% after 60 days of incubation period. In case of 50% RH the total percentage incidence observe 3.48% after 60 days of storage and this percentage incidence is increased continuously to 8.29%. Under 96% RH the percentage incidence observe while after 15, 30, 45 days they show incidences 2.77, 4.60, 7.56 and 9.80%, respectively. Under 100% RH incidence occur 4.31, 6.79, 9.98 and 13.17% after 15, 30, 45 and 60 days of storage period. The fungi *F. solani*, *A. niger*, *Papulaspora immerse* observe after 15 days of incubation and percentage incidence from 2.53, 0.35 and 0.11% after 60th days at 30% RH, increase to 3.83, 2.06 and 0.53% on 60th days under 100% RH. Inspect *A. parasiticus* which only observe under 100% RH, other fungi include: *F. oxysporum*, *F. lateritum*, *F. reticulatum*, *A. terreus*, *Sporothrix* sp., *A. fumigatus*, *Achlya* sp., *Nigrospora oryzae*, *N. sphaericha*, *Penicillium* sp., *Curvularia lunata*, *Monilia sitophila* and *Chaetomium spirale* are observed under 50, 75, 96 and 100% RH. *A. terreus* only observe under 75, 96 and



Graph 1: Percentage incidence of mycoflora associated with the root of *Clerodendrum phlomoides* (Fresh samples)



Graph 2: Percentage incidence of mycoflora associated with the root of *Clerodendrum phlomoides* (market samples)

Table1: Percentage incidence of fungal isolated from the fresh drug roots of *C. phlomoides* stored at various relative humidity

Mycoflora	con	30%				50%				75%				96%				100%			
		15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60
<i>F. solani</i>	0.59	0.23	0.64	2.42	2.53	0.64	1.06	2.18	2.65	0.76	1.59	2.53	2.77	1.65	2.30	2.77	3.13	2.18	2.77	2.95	3.83
<i>F. oxysporum</i>	0.05	-	-	-	0.05	-	0.05	0.41	0.47	0.05	0.05	1.06	1.35	0.05	0.17	1.18	1.59	0.05	0.53	1.59	1.33
<i>F. lateritum</i>	-	-	-	-	-	-	-	0.05	0.11	-	-	0.05	0.17	-	0.05	0.11	0.23	0.05	0.11	0.23	0.41
<i>F. reticulatum</i>	-	-	-	-	-	-	-	-	0.05	-	-	-	0.05	-	-	0.05	0.11	-	-	0.11	0.23
<i>A. niger</i>	0.29	0.05	0.05	0.23	0.35	0.11	0.23	1.18	1.47	0.53	0.04	1.65	1.89	0.64	1.24	1.53	1.94	0.82	1.35	1.65	2.06
<i>A. terreus</i>	-	-	-	-	-	-	-	-	-	-	-	0.05	0.11	-	-	0.11	0.23	0.05	-	0.23	0.29
<i>Sporothrix</i> sp.	-	-	-	-	-	-	-	-	0.05	-	0.05	0.05	0.05	-	0.05	0.11	0.05	0.05	0.11	0.23	0.29
<i>Aspergillus fumigatus</i>	-	-	-	-	-	-	-	0.05	0.17	0.05	-	0.11	0.23	0.05	0.11	0.17	0.23	0.05	0.11	0.29	0.35
<i>Alternaria</i> sp.	-	-	-	0.05	0.05	-	-	0.11	0.11	-	0.05	0.11	0.17	0.05	0.11	0.23	0.23	0.11	0.17	0.29	0.41
<i>Achlya</i> sp.	-	-	-	0.05	0.11	-	-	0.05	0.05	0.05	0.05	0.05	0.17	0.05	0.05	0.11	0.17	0.11	0.11	0.17	0.29
<i>Nigrospora oryzae</i>	-	-	-	-	-	-	0.05	0.17	0.17	-	0.05	0.11	0.17	0.05	0.05	0.11	0.17	0.17	0.17	0.23	0.41
<i>N. sphaericha</i>	-	-	-	-	0.05	-	-	0.05	0.11	-	-	0.17	0.17	-	0.05	0.23	0.23	0.05	0.23	0.29	0.35
<i>Penicillium</i> sp.	-	-	-	-	-	-	-	-	-	-	-	0.05	0.05	-	-	0.05	0.17	-	0.05	0.11	0.29
<i>Papulaspora immerse</i>	0.17	-	0.05	0.05	0.11	0.05	0.05	0.05	0.17	0.05	0.05	0.17	0.29	0.05	0.11	0.23	0.41	0.11	0.17	0.41	0.53
<i>Curvularia lunata</i>	-	-	-	0.05	0.11	-	0.05	0.05	0.23	-	-	0.05	0.17	0.05	0.05	0.11	0.17	0.05	0.11	0.23	0.29
<i>Monilia sitophila</i>	-	-	-	-	-	-	0.05	0.05	0.05	-	0.05	0.11	0.17	0.05	0.05	0.17	0.29	0.05	0.23	0.29	0.35
<i>Chaetomium spirale</i>	-	-	-	-	0.05	-	-	0.05	0.05	-	0.05	0.11	0.17	-	0.11	0.17	0.29	0.23	0.35	0.53	0.64
<i>Aspergillus parasiticus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	0.05	0.05	0.11	0.17
Total	1.18	0.29	0.76	2.89	3.48	0.82	1.59	4.54	6.02	1.53	3.07	6.55	8.26	2.77	4.60	7.56	9.80	4.31	6.79	9.98	13.17

Table 2: Percentage incidence of fungal isolated from the market drug roots of *C. phlomoides* stored at various relative humidity

Mycoflora	con	30%				50%				75%				96%				100%			
		15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60
<i>Aspergillus niger</i>	0.51	0.51	0.51	0.72	1.03	0.51	0.62	0.82	1.37	0.51	0.72	1.13	1.50	0.72	0.93	1.24	2.17	1.03	1.50	1.75	3.61
<i>Aspergillus fumigatus</i>	0.20	0.10	0.20	0.31	0.41	0.20	0.41	0.51	0.72	0.31	0.82	0.93	1.24	0.41	0.72	1.5	1.75	0.51	0.82	1.75	2.068
<i>Aspergillus flavus</i>	0.51	0.10	0.20	0.41	0.51	0.10	0.31	0.72	0.93	0.20	0.41	1.03	1.24	0.31	0.51	0.82	1.5	0.72	0.93	1.86	2.17
<i>A. terreus</i>	-	-	-	-	-	-	-	0.10	0.20	-	0.10	0.20	0.41	0.10	0.31	0.51	0.72	0.41	0.51	0.93	1.24
<i>Rhizopus oryzae</i>	0.10	0.10	0.10	0.20	0.31	0.10	0.20	0.52	0.62	0.10	0.31	0.51	0.93	0.41	0.51	0.82	0.93	0.72	0.93	1.5	1.96
<i>Papulaspora immerse</i>	-	-	-	0.10	-	0.10	0.20	0.31	0.41	-	0.31	0.41	0.51	0.10	0.41	0.72	0.93	0.51	0.93	1.13	1.5
<i>Chaetomium</i> sp.	-	-	-	-	-	-	-	-	-	-	-	0.31	0.51	-	0.31	0.71	0.93	0.10	0.31	0.72	1.50
<i>Chaetomium globosum</i>	-	-	-	-	-	0.10	-	-	0.10	0.20	-	0.10	0.20	0.31	0.20	0.41	0.51	0.82	0.31	0.72	0.93
<i>Taeniolella alta</i>	-	-	-	-	-	-	-	-	0.10	-	-	0.10	0.20	-	0.10	0.51	0.72	-	0.31	0.72	1.03
<i>Stemphylium paxianum</i>	-	-	-	-	-	-	-	-	-	-	-	-	0.10	-	-	0.10	0.20	-	0.20	0.31	1.5
<i>Nigrospora oryzae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.10	0.20	-	-	0.31	0.51
<i>Aphanomyces</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.10	-	-	0.20	0.51
<i>Penicillium</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.31	0.51
Total	1.32	0.81	1.01	1.74	2.26	1.01	1.74	2.98	4.45	1.32	2.67	3.7	6.33	2.05	4	7.44	10.6	4.82	6.75	12.2	19.03

In case of market samples, total percentage incidence of different fungi recorded (Table 2), which after 15 days of incubation under 30, 50, 75, 96 and 100% RH, observe 0.81, 1.01, 1.32, 2.05 and 4.82 % incidence which increase to 2.26, 4.45, 6.33, 10.66 and 19.03% after 60 days of storage period. The total percentage incidence of fungi *A. niger*, *A. fumigatus*, *A. flavus*, *Rhizopus oryzae*, *Chaetomium* sp. and *Papulaspora immerse* observe under all tested relative humidities while *A. terreus*, *Chaetomium globosum* and *Taeniolella alta* occur under 50, 75, 96 and 100% RH. The incidence of *Nigrospora oryzae*, *Aphanomyces* sp. and *Stemphylium paxianum* observe only under 96 and 100% RH. The fungi *Penicillium* sp. only under 100% RH and after 45 days of incubation observe.

DISCUSSION

Christensen (1957) grouped fungi into two categories: field fungi and storage fungi. This division is not taxonomically valid but is based primarily upon moisture requirements. Field fungi attack developing and mature tissue of herbal drugs; storage fungi are usually encountered when organs of medicinal plants are stored after harvest. For the most part, the above categories are accurate; however, exceptions exist, *A. flavus* can invade in the field and *Fusarium* can continue to decay of plant tissues in storage if the moisture is high enough (Caldwell and Tuite, 1974). There is a correlation between the growth and incidence of fungi with high relative humidity and prolonged incubation days. The predominant fungi associated with drug roots *Fusarium* and *Aspergillus* species which both are toxigenic fungi and under suitable condition can invade to tissue plants and produce toxin, which consumption of these contaminated materials by human leads to several physiological disorders and even death. These observations are inconformity with the other works of Aziz *et al.* 1998; Singh and Gupta, 1982; Sinha and Sinha, 1988; Heiberg and Ramsey, 1953. Therefore, there is an urgent need to prevent the entrance of such contaminated crude drug samples into commercial herbal drugs.

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