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NATURAL OCCURRENCE OF MYCOFLORA AND DETERIORATION OF TOTAL ALKALOIDS AND GLYCOSIDES AMOUNTS IN *STEREOSPERMUM CHELONOIDES* DC. ROOTS UNDER STORAGE

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ABSTRACT: In the present study, total 15 fungal species isolated from roots of drug *Stereospermum chelonoides* DC. such as: *Fusarium solani*, *F. lateritum*, *F. semitectum*, *F. oxysporum*, *Aspergillus ochraceus*, *A. niger*, *A. terreus*, *Theilavia terricola*, *Papulaspora immerse*, *Scytallidium thermophilum*, *Didymostilbe* sp., *Cunningamella elegans*, *Aphanomyces* sp., *Sordaria fimicola* and *Mucor praini.* Species of *Fusarium* showed maximum percentage incidence. The root samples stored at different relative humidities 30, 50, 75, 96 and 100% RH. Quantitative estimation of alkaloids and glycosides in association with isolated fungi was done. Percentage incidence of storage fungi and the rate of deterioration of chemical constituents in the samples were noted at above 75% RH. Analysis of variance also showed the effect of relative humidity and incubation days on biodeterioration of these chemical constituents were significant at 5% level of significance.

INTRODUCTION: *Stereospermum chelonoides* DC. (Syn: *S. suaveolens*) is commonly called as "Patla", "Atkapali", "Paruli", "Gachh" and "Padri". It belongs to the "Bignoniacea" family. Barks, flowers, roots and leaves of *S. suaveolens* are used by traditional healers, rural communities and pharmaceutical companies for remedies of diseases like heating, vomiting, eructation, piles, acidity, diarrhoea, gonorrhoea, loss of taste, malaria and other fevers [1].

The roots are an ingredient of Dashmoola and it is regarded as cooling, astringent cardiotonic, bitter, diuretic and tonic and generally used in combination with other medicine. The ashes of this plant are used in the preparation of alkaline water and caustic. Medicinal plants may be associated with a broad variety of microbial contaminants, represented by bacteria, fungi and viruses. Inevitably, this microbiological background depends on several environmental factors, relative humidity and temperature have the most influence on the growth of fungi. There are a few reports concentrating on the subject mycoflora associated with herbal drugs and changes in their chemical constituents due to spoilage of fungi, therefore, attempt had been made to study mycoflora associated with roots of *S. chelonoides* from the field at different relative humidity (RH).

MATERIAL AND METHODS: The fresh roots of drug *S. chelonoides* were collected in healthy, flowering and fruiting conditions from different localities of Maharashtra, India. For avoiding aerial contamination, samples were brought to the laboratory in polyethylene bag. Blotter test and agar plate method as recommended by International Seed Testing Association . were done for isolation of mycoflora associated with roots. Then samples sterilized with 2% sodium hypochlorite solution and thoroughly washed with sterilized distilled water. For evaluation of biodeterioration of alkaloids and glycosides contents related to mycoflora, the root samples were stored in small muslin clothes at 30, 50, 75, 96 and 100 % RH for

90 days in the room temperature. The root samples were taken out internal 15, 30, 45, 60, and 75 and 90 days, thoroughly washed with distilled water and plated in Petri plates. The percentage incidence of mycoflora was recorded from first day to 60th day of storage. Fungi were identified by using different keys [3-5]. Some parts of washed root samples were dried in oven and powdered by grinder and were used for biochemical analysis by standard methods [6-7].

RESULTS: During the course of present study total 15 fungi species were recorded. They include: *Fusarium solani*, *F. lateritum*, *F. semitectum*, *F. oxysporum*, *Aspergillus ochraceus*, *A. niger*, *A. terreus*, *Theilavia terricola*, *Papulaspora immerse*, *Scytallidium thermophilum* *Didymostilbe* sp., *Cunningamella elegans*, *Aphanomyces* sp., *Sordaria fimicola* and *Mucor praini*. In general, there was an increase in fungal incidence with the length of storage period (**Table 1**).

The fresh roots of *Stereospermum chelonoides* stored under 30, 50, 75, 96 and 100 % RH. Total percentage incidence of fungi after 15 days of incubation observed 0.46%, this amount increased to 2.97% after 60 days of storage. Under 50% RH, from 15 days to 60 days total percentage incidence occurred 0.4% to 3.91%.

In the case of 75% RH, this amount from 1.09% increased to 8.98%, under 96 and 100% RH total percentage incidence of fungi after 15 days observed 1.25% and 2.66 %, these percentages incidences increased to 13.54 % and 20.97% after 60 days of incubation.

TABLE 1: PERCENTAGE INCIDENCE OF MYCOFLORA ISOLATED FROM THE DRUG ROOTS OF STEREOSPERMUM CHELONOIDES 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
F. solani	0.15	-	0.15	0.47	0.63	-	0.47	0.79	0.47	0.31	0.79	1.42	1.26	-	1.10	1.74	1.74	0.79	1.42	2.37	2.84
F. lateritum	-	-	-	-	-	-	-	-	-	-	-	0.15	0.31	-	-	0.31	0.47	-	0.15	0.47	0.79
F. semitectum	0.79	0.31	0.47	0.62	0.63	0.15	0.79	1.10	0.94	0.47	0.63	1.74	1.42	0.63	0.79	2.84	2.84	0.79	1.42	3.006	3.32
F. oxysporum	0.94	0.15	0.15	0.15	0.47	0.15	0.15	0.31	1.10	0.31	0.47	0.47	1.74	0.47	0.79	1.26	2.37	0.47	0.94	1.42	5.063
Aspergillus niger	-	-	-	-	0.47	-	-	-	0.31	-	-	0.15	0.79	-	-	0.31	1.42	0.15	0.31	0.31	1.58
A. ochraceus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.15	0.15	0.15
A. terreus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.15	-	0.31	-	-	0.15	0.47
Theilavia terricola	-	-	-	0.31	0.47	0.15	-	0.47	0.63	-	0.15	1.10	0.79	0.15	0.31	1.42	1.74	0.31	0.47	1.58	2.37
Papulaspora immerse	-	-	-	-	0.15	-	0.15	-	0.31	-	-	-	0.47	-	0.31	0.15	0.63	-	-	0.31	0.79
Didymostilbe sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.15	0.31
Cunningamella elegans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.15	0.15	-	0.15	0.47	0.31
Aphanomyces sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.15	-	-	-	0.15
Sordaria fimicola	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.15	0.15	-	-	-	0.15
Mucor praini	-	-	-	-	-	-	-	-	-	-	-	-	1.10	-	0.15	0.15	0.15	-	0.15	0.15	0.31
Scytallidium thermophilum	-	-	-	0.15	0.15	-	-	0.15	0.15	-	-	-	1.10	-	-	0.15	1.42	0.15	0.31	0.79	2.37
Total	1.88	0.46	0.77	1.7	2.97	0.45	1.56	2.82	3.91	1.09	2.04	5.3	8.98	1.25	3.6	8.63	13.54	2.66	5.47	11.32	20.97

However the increase in fungal growth caused decreasing in total alkaloids and glycosides amount (**Table 2 and 3**).

The roots of *S. chelonoides* stored under higher relative humidities (96 and 100%) and maximum storage period after 60th days showed maximum deterioration in total alkaloids and glycosides content.

The control samples of *S. chelonoides* contained 2.89% total alkaloids. Deterioration of alkaloids is observed under 30% RH, 2.8, 2.73, 2.68, 2.41, 2.28 and 2.09 % alkaloids after the storage of 15, 30, 45, 60, 75 and 90 days respectively.

Similarly, decrease in alkaloids content in the root samples under 100 % RH 2.62, 2.42, 2.30, 2.001, 1.79 and 1.63 % observed after storage of 15, 30, 45, 60, 75 and 90 days, respectively.

TABLE 2: DETERIORATION OF TOTAL ALKALOIDS CONTENT (MG/100MG) IN ROOT OF *STEREOSPERMUM CHELONOIDES* (FRESH AND MARKET SAMPLES) AT DIFFERENT RELATIVE HUMIDITIES

Incubation days	Control	30%	50%	75%	96%	100%
1 day	2.89±0.061	2.89±0.061	2.89±0.061	2.89±0.061	2.89±0.061	2.89±0.061
15days	2.89±0.055^{b}	2.8±0.015^{b}	2.79±0.055^{a}	2.72±0.01^{a}	2.69±0.02^{a}	2.62±0.052^{a}
30days	2.88±0.011^{b}	2.73±0.045^{b}	2.71±0.02^{b}	2.6±0.025^{b}	2.51±0.037^{a}	2.42±0.041^{a}
45 days	2.87±0.011^{d}	2.68±0.036^{c}	2.6±0.03^{c}	2.5±0.056^{c}	2.4±0.020^{b}	2.3±0.01^{a}
60 days	2.87±0.047^{c}	2.41±0.062^{c}	2.3±0.055^{b}	2.2±0.02^{b}	2.09±0.083^{a}	2.001±0.045^{a}
75 days	2.87±0.050^{c}	2.28±0.064^{b}	2.15±0.045^{b}	2.12±0.075^{ab}	1.9±0.10^{ab}	1.79±0.055^{a}
90 days	2.89±0.040^{c}	2.09±0.083^{c}	1.99±0.010^{c}	1.9±0.068^{c}	1.74±0.046^{b}	1.63±0.040^{a}

Data are the mean of three replicates ± standard deviation. P- Value denoted the significance of differences between the mean by univariate comparison statistics. The value followed by different letters differ significantly by Duncan's multiple rang test at P=Sig= 0.05

The control of fresh samples contained 6.71% total glycosides and these percentages incidences reduced to 3.70% under influence of different RH and different incubation days. The drug under 30 and 50 % RH showed minimum deterioration of total glycosides but in case of 75% RH showed 6.49, 6.37, 5.91, 5.71, 5.1% after 15, 30, 45, 60 , 75 and 90 days of incubation. In case of 96 % RH also observed the deterioration of glycosides 6.37, 6.31, 5.87, 5.41, 4.71, 3.88% after 15, 30, 45, 60, 75 and 90 days of storage. Lastly maximum deterioration observed under 100 % RH, after the incubation period of 15, 30, 45, 60, 75 and 90 days of storage 6.28, 6.18, 5.60, 4.40, 4.15, 3.7% respectively.

TABLE 3: DETERIORATION OF TOTAL GLYCOSIDES CONTENT (MG/100MG) IN ROOT OF *STEREOSPERMUM CHELONOIDES* (FRESH AND MARKET SAMPLES) AT DIFFERENT RELATIVE HUMIDITIES

Incubation days	Control	30%	50%	75%	96%	100%
1 day	6.71±0.015	6.71±0.015	6.71±0.015	6.71±0.015	6.71±0.015	6.71±0.015
15days	6.71±0.23^{d}	6.69±0.055^{c}	6.52±0.052^{c}	6.49±0.05^{b}	6.37±0.051^{a}	6.28±0.052^{a}
30days	6.71±0.015^{c}	6.48±0.02^{c}	6.41±0.072^{d}	6.37±0.051^{bc}	6.31±0.052^{ab}	6.18±0.047^{a}
45 days	6.71±0.030^{d}	6.37±0.051^{c}	6.11±0.047^{c}	5.91±0.06^{bc}	5.87±0.052^{b}	5.60±0.011^{a}
60 days	6.7±0.050^{d}	6.09±0.056^{cd}	5.89±0.10^{c}	5.71±0.015^{b}	5.41±0.11^{b}	4.40±0.015^{a}
75 days	6.69±0.065^{c}	5.71±0.20^{c}	5.40±0.12^{c}	5.01±0.14^{b}	4.71±0.05^{b}	4.15±0.045^{a}
90 days	6.67±0.025^{c}	5.1±0.17^{c}	4.71±0.17^{b}	4.28±0.15^{b}	3.88±0.15^{a}	3.7±0.15^{a}

Data are the mean of three replicates ± standard deviation. P- Value denoted the significance of differences between the mean by univariate comparison statistics. The value followed by different letters differ significantly by Duncan's multiple rang test at P=Sig= 0.05

The results were analyzed statistically which analysis of variance showed that relative humidities and incubation days on the deterioration of alkaloids and glycosides contents were significant at 5% &1% level of significance, P value (sig) < 0.001, 0.005.

DISCUSSION: Deterioration of alkaloids and glycosides amounts under different relative humidities due to spoilage of fungi which might be due to its degradation into simpler forms of chemical constituents by enzymes which produce by fungi and their utilization as a source of energy for their growth. This was in accordance with the result of earlier workers [8-10].

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