

Research Article

Studies on Mycoflora Associated with Market Roots of Drug *Solanum indicum* Vent. and their Effect on Changes of Chemical Constituents under Storage

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ABSTRACT

In the present study the market roots of drug plant *Solanum indicum* Linn. were selected for isolation of mycoflora associated with market roots and changes in chemical constituents amount under storage of different relative humidity and incubation days due to spoilage of fungal contamination. Isolation and identification of fungi and phytochemical analysis were done according standard methods. The results showed that total 12 fungi were associated with market root drug. *A. niger* showed highest percentage incidence while *Stemphylium paxianum* and *Alternaria* sp. observed in minimum percentage incidence. The drug stored under influence of different relative humidities viz. 30, 50, 75, 96 and 100% and different incubation days 15, 30, 45, 60 days. Quantitative estimation of chemical constituent's amount such as sugars, proteins, phenols, alkaloids and glycosides in relation to association of fungi was done. The results indicated that maximum growth and percentage incidence of fungi as well as biodeterioration of chemical constituents observed under high relative humidities 75, 96 and 100% RH and long storage periods 45 and 60 days. Analysis of variance also showed that the effect of relative humidity and incubation days on biodeterioration of chemical constituents amount were significant.

Key words: chemical constituents, deterioration, fungi, percentage incidence.

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INTRODUCTION

The plant *Solanum indicum* Linn. belongs to Solanaceae family commonly called as "Brahanta". This plant has been used for centuries in India, both for dietary purposes and in the Ayurvedic medicinal tradition. It is regarded as diuretic, useful in dropsy and expectorant, useful in cough and catarrhal affections, also diaphoretic and stimulant. Fruit and roots contain wax, fatty acids and alkaloids Solanine and Solanidine [1]. The roots are one of the ingredients of Dashmoola Kautha of Hindi medicine. It is seldom used alone. The roots take internally manifests strongly exciting qualities.

It is employed in difficult parturition and in toothache. It is also used in fever, work complaints and colic [2]. Roots are bitter, acrid, astringent, thermogenic, anodyne, digestive, carminative anthelmintic, stomachic, constipating, resolving, demulcent, depurative expectorant,

aphrodisiacs, febrifuge and cardio tonic [3]. 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 and exerts an important impact on the overall quality of herbal products and preparations. Therefore, it has been decided to study associated mycoflora and deterioration of chemical constituents such as sugars, phenols, proteins, alkaloids and glycosides in relation to spoilage of fungi in market roots of drug *S. indicum* under storage of different relative humidity 30, 50, 75, 96 and 100 % and incubation days 15, 30, 45, 60, 75 and 90.

MATERIALS AND METHODS

The market roots of *S. indicum* were collected from different Kashtaushadhi shopkeepers. It was brought to the laboratory in separate polyethylene bags

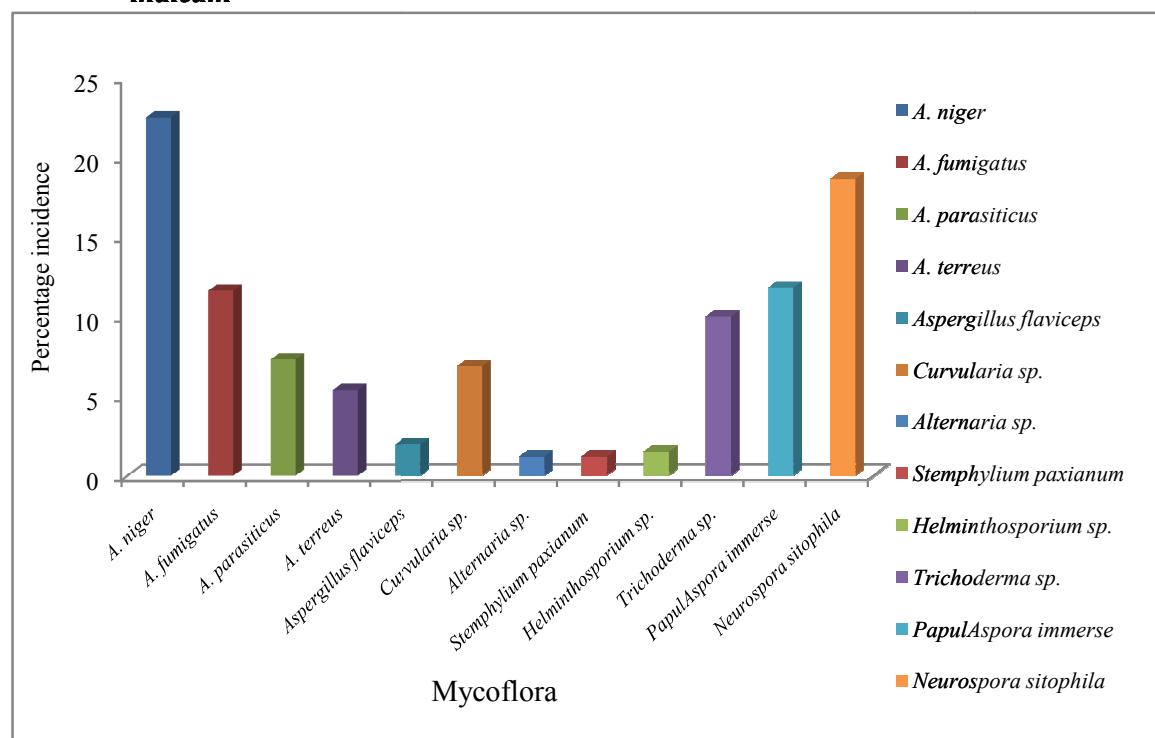
to avoid aerial contamination. Blotter test method, as recommended by International Seed Testing Association [4] was adopted for isolation of fungi. Agar plate method was also used. The roots were sterilized with 2% NaOCl solution before plating. In order to evaluate the chemical changes in relation to associated fungi, the samples were stored in small muslin cloth bags under different RH levels 30, 50, 75, 96, 100 % and at $28 \pm 3^\circ\text{C}$ temperature for 90 days. At an interval of 15 days, samples were taken out and washed thoroughly with sterilized distilled water and plated in Petri plates. The isolation of mycoflora was recorded from first day to 60th day of storage. Fungi were identified by using references [6-10]. Some parts of samples after washing with distilled water were dried in oven for chemical analysis. Chemical analysis were estimated by the procedure described by Lowry *et al.* [11] for total protein, Singh *et al.* [12] for total phenols, Harborne [13] for total alkaloids and Kokate *et al.* [14] for glycosides. Anthrone methods for

Total sugars (TS) and Dinitrosalicylic acid (DNSA) method for Reducing sugar (RS) amount [15] were also followed. Simple correlation were run between selected parameters using Statistical Package for Social Science (SPSS) software in which statistical significance was determined at 0.05 % probability levels.

RESULTS

Total 12 fungi were isolated from the market roots of *Solanum indicum* in varying percentages (Graph 1) such as *A. niger* (22.53%), *A. fumigatus* (11.64%), *A. parasiticus* (7.31%), *Aspergillus flaviceps* (1.96%), *Curvularia lunata* (6.86%), *Alternaria* sp. (1.19%), *Stemphylium paxianum* (1.19%), *Helminthosporium* sp. (1.49%), *Trichoderma* sp. (10%), *Papulaspora immerse* (11.79%), *Neurospora sitophila* (18.65%). It was observed that *A. niger* following by *Neurospora sitophila*, *Papulaspora immerse* and *A. fumigatus* showed high percentage incidence while *Helminthosporium* sp., *Stemphylium paxianum* and *Alternaria* sp. observed in less percentage incidence.

Figure 1: Percentage incidence of mycoflora associated with market root of *Solanum indicum*



Market roots of *S. indicum* stored under different relative humidity and percentage incidence of isolated fungi recorded (**Table 1**). Total percentage incidence of all fungi after 15 days under 30 and 50 % RH observed 0.99 and 1.29 % which they increased to 3.21 and 5.16 % after 60 days of incubation. In cases of 75, 96 and 100 % RH, after 15 days of storage observed 1.58, 2.62 and 4.12 %, after 60 days they increased to 8, 10.97 and 17.08 %, respectively. Different species of *Aspergillus* like *A. niger*, *A. fumigatus*, *A. parasiticus*, *A. terreus* and *Aspergillus flaviceps* isolated and observed under higher relative humidity. Under 100% RH and after 60 days of incubation, *A. niger* with 2.68% showed the highest percentage incidence. *A. fumigatus*, *A. parasiticus*, *Papulaspora immerse* and *Neurospora sitophila* with same percentage incidence 2.33 % observed. After them *Trichoderma* sp., *A. terreus*, *Curvularia lunata* observed with 1.34, 1.19 and 1.04 % incidence. *Alternaria* sp., *Stemphylium paxianum* and *Helminthosporium* sp. observed in less percentage incidence under only 96 and 100% RH. The genus *Aspergillus* sp. also observed in very less percentage incidence.

Deterioration of selected chemical constituents such as sugars (Total sugar and reducing sugar), proteins, phenols, alkaloids and glycosides in relation to associated fungi recorded.

Amount of total sugars (TS) and reducing sugars (RS) in market sample of this drug at the first day showed 40.23 and 21.58 % (**Table 2**), these values gradually decreased, while after 90 days of incubation in each relative humidity maximum deterioration observed, 32.2, 14.52% (30 % RH); 15.65, 13.61% (50 % RH); 14.98, 11.85 % (75 % RH); 13.55, 10.45 (96 % RH) and 12.07, 10 % (100 % RH).

In the case of changes in total protein amounts, at the first day samples contained 19.02% but after 30, 60 and 90 days of incubation deteriorated to 18.33, 16.38, 15.13 % and 17.77, 15.83, 14.44 % under 30 % and 50 % RH (**Table 3**). At 96 and 100 % RH, total proteins value showed deterioration 16.25, 12.91, 13.055% and

15.27, 12.36 and 11.11 % after 30, 60 and 90 days of storage respectively.

Control sample of drug *S. indicum* contained 7.08 % total phenols which after 15 days of incubation under 100 % RH increased to 6.96 % but after that gradually deteriorated with increasing incubation period and relative humidity (**Table 4**). After 30, 60 and 90 days of incubation period total phenols value were 6.88, 6.51, 6.25 % (30 % RH); 6.45, 6.31, 5.84 % (50 % RH); 6.31, 6.12, 5.15 % (75 % RH), 6.25, 5.88, 4.68 % (96 % RH) and 6.20, 5.55, 4.15% (100 % RH).

Reduction in total alkaloid amounts also observed, control sample of this drug showed 12.20 %, but after 90 days of incubation period under 30, 50, 75, 96 and 100 % RH, deteriorated to 11.86, 11.65, 11.48, 11.42 and 11.31 %, respectively (**Table 5**).

Amount of total glycosides in samples at the first day showed 9.75%, this value gradually decreased, while after 90 days of incubation in each relative humidity maximum; deterioration observed to 8.29% (30 % RH); 8.086% (50% RH); 8.04% (75% RH); 7.81% (96 % RH) and 7.73 % (100% RH) (**Table 6**).

DISCUSSION

The result of this investigation indicated that the maximum reduction of chemical constituents was after 60th to 90th days of incubations while total percentage incidence of fungi were also maximum in percentage incidence of mycoflora associated with market samples. The principal factors on fungal growth and deterioration are moisture, temperature, atmosphere, aeration, PH, condition of the storage and incubation period of herbal plants. All of these factors interact as deterioration progresses, but moisture and temperature were probably most important. If the moisture content is maintained at a sufficiently low level, medicinal plant can be stored for long times with less deterioration even under otherwise unsuitable storage conditions. Storage fungi are predominantly species of *Aspergillus* and *Penicillium* [16]. Several workers have been showed deterioration of chemical constituents under storage due to spoilage of fungi in different plants [17- 20].

Table 1: Percentage incidence of fungal isolated from market root of *Solanum indicum* 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>	0.29	0.29	0.44	0.74	1.19	0.44	0.59	0.74	1.64	0.44	0.74	1.04	1.79	0.74	0.89	1.34	2.089	0.89	1.49	1.94	2.68
<i>Aspergillus fumigatus</i>	0.14	0.14	0.14	0.14	0.29	0.14	0.14	0.29	0.44	0.14	0.29	0.44	0.74	0.29	0.59	0.89	1.19	0.44	1.04	1.34	2.23
<i>A. parasiticus</i>	0.14	0.14	0.14	0.14	0.29	0.14	0.14	0.29	0.44	0.14	0.29	0.44	0.74	0.29	0.59	0.89	1.19	0.44	1.04	1.34	2.23
<i>A. terreus</i>	-	-	-	-	0.14	-	-	0.14	0.29	-	0.14	0.29	0.44	0.14	0.14	0.29	0.59	0.29	0.44	0.74	1.19
<i>Aspergillus flaviceps</i>	-	-	-	-	0.14	-	-	-	0.14	-	-	0.14	0.14	-	-	0.14	0.29	-	0.14	0.29	0.44
<i>Trichoderma</i> sp.	0.14	0.14	0.14	0.14	0.29	0.14	0.14	0.44	0.59	0.14	0.29	0.59	0.89	0.29	0.44	0.74	1.04	0.44	0.59	0.89	1.34
<i>Papulaspora immerse</i>	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.29	0.44	0.14	0.44	0.74	0.89	0.29	0.44	0.74	1.04	0.74	0.89	1.34	2.23
<i>Neurospora sitophila</i>	0.14	0.14	0.29	0.44	0.59	0.29	0.59	0.74	0.89	0.44	0.74	1.04	1.64	0.44	0.89	1.49	1.94	0.59	1.19	1.79	2.23
<i>Curvularia lunata</i>	-	-	-	0.14	0.14	-	0.14	0.14	0.29	0.14	0.29	0.44	0.59	0.14	0.44	0.59	0.74	0.29	0.44	0.74	1.044
<i>Alternaria</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	0.14	-	-	-	0.29	-	-	0.29	0.44
<i>Stemphylium paxianum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.29	-	0.14	0.29	0.44
<i>Helminthosporium</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.14	0.29	-	0.14	0.29
Total	0.99	0.99	1.29	1.88	3.21	1.29	1.88	3.07	5.16	1.58	3.22	5.16	8	2.62	4.42	7.25	10.97	4.12	7.54	11.28	17.08

Table 2: Deterioration of Total sugars (TS) and Reducing sugars (RS) content (mg/100 mg) in root of *Solanum indicum* at different relative humidities

Incubation days	Control		30%		50%		75%		96%		100%	
	TS	RS	TS	RS	TS	RS	TS	RS	TS	RS	TS	RS
1 day	40.23 ±1.02	21.58 ±0.13	40.23 ±1.02	21.58 ±0.13	40.23 ±1.02	21.58 ±0.13	40.23 ±1.02	21.58 ±0.13	40.23 ±1.02	21.58 ±0.13	40.23 ±1.02	21.58 ±0.13
15 days	40.23 ±0.31 ^c	21.58 ±0.045	37.79 ±0.19 ^c	20.36 ±0.069	36.27 ±0.31 ^c	19.14 ±0.090	32.32 ±2.21 ^b	18.86 ±0.18 ^b	29.88 ±1.92 ^a	17.51 ±0.48 ^a	26.09 ±0.73 ^a	17.23 ±0.094 ^a
30 days	40.23 c	21.58 ±0.072	37.79 ±1.13 ^c	19.14 ±0.11 ^c	35.31 ±1.99 ^{bc}	18.86 ±0.23 ^{bc}	32.11 ±1.99 ^b	18.009 ±0.19 ^{ab}	28.61 ±0.95 ^a	16.78 ±0.19 ^a	20.41 ±0.69 ^a	15.83 ±0.30 ^a
45 days	40.23 c	21.58 ±0.19 ^c	36.86 ±0.045	18.46 ±0.88 ^c	31.06 0.23 ^c	17.78 ±1.65 ^c	27.18 ±0.25 ^{bc}	16.78±0 ±1.56 ^{abc}	25.50 .53 ^{ab}	15.29 ±2.46 ^a	19.40 ±0.49 ^{ab}	14.84 ±2.63 ^a
60 days	40.23 ±0.19 ^c	21.58 ±0.13+	34.84 ±9.40 ^c	17.11 ±0.11 ^c	24.36 ±0.63 ^c	16.47 ±0.56 ^{bc}	21.71 ±2.07 ^{abc}	15.83 ±0.29 ^{ab}	17.8± 1.002 ^a	14.43 ±0.26 ^a	16.11 ±0.82 ^a	13.57 ±0.19 ^a
75 days	40.23 c	21.58 ±0.29 ^c	33.5 ±11.73 ^c	15.88 ±0.45 ^c	17.34 ±0.81 ^c	15.52 ±0.22 ^c	17.29 ±0.33 ^{bc}	14.34 ±0.2 ^{bc}	16.58 ±0.14 ^a	13.07 ±0.45 ^{ab}	14.68 ±1.75 ^a	12.35 ±0.21 ^a
90 days	40.23 c	21.58 ±0.072	32.2 ±13.74 ^c	14.52 ±0.60 ^c	15.65 ±0.45 ^{abc}	13.61 ±0.81 ^{ab}	14.98 ±0.19 ^{ab}	11.85 ±0.26 ^{ab}	13.55 ±0.59 ^a	10.45 ±0.56 ^a	12.07 ±0.64 ^a	10 ±0.30 ^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

Table 3: Deterioration of proteins content (mg/100 mg) in market root of *Solanum indicum* at different relative humidities

Incubation days	control	30%	50%	75%	96%	100%
1 day	19.02±0.08	19.02±0.08	19.02±0.08	19.02±0.08	19.02±0.08	19.02±0.08
15 days	19.02±0.21 ^d	18.75±0.16 ^d	18.61±3.61 ^c	18.05±0.36 ^b	16.80±0.60 ^b	16.25±0.21 ^a
30 days	19.02±0.27 ^d	18.33±0.16 ^c	17.77±0.80 ^c	16.38±0.21 ^b	16.25±0.34 ^b	15.27±0.36 ^a
45 days	19.02±0.34 ^d	16.94±0.50 ^d	16.25±0.34 ^c	15.27±0.60 ^{bc}	14.30±0.48 ^b	13.61±0.60 ^a
60 days	19.02±0.13 ^d	16.38±0.24 ^d	15.83±0.28 ^c	13.88±0.21 ^b	12.91±0.50 ^b	12.36±0.21 ^a
75 days	19.02±3.24 ^c	15.83±0.24 ^a ^b	15.68±0.48 ^a ^b	15.23±1.57 ^a	14.02±1.004 ^a	13.47±1.18 ^a
90 days	19.02±0.08 ^c	15.13±2.16 ^b	14.44±0.21 ^b	13.05±0.48 ^a ^b	13.055±0.48 ^a	11.11±0.16 ^a ^b

Data in tables 3,4,5 and 6 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

Table 4: Deterioration of total phenols content (mg/100 mg) in market root of *Solanum indicum* at different relative humidities

Incubation days	Control	30%	50%	75%	96%	100%
1 day	7.08±0.049	7.08±0.049	7.08±0.049	7.08±0.049	7.08±0.049	7.08±0.049
15 days	7.08±0.059 ^c	7.08±0.011 ^b	6.85±0.030 ^a	6.86±0.11 ^a	6.85±0.13 ^a	6.96±1.17 ^a
30 days	7.08±0.040 ^c	6.88±0.039 ^b	6.45±0.28 ^b	6.31±0.14 ^{ab}	6.25±0.049 ^a	6.20±0.049 ^a
45 days	7.08±0.049 ^d	6.73±0.030 ^d	6.25±0.15 ^c	6.18±0.093 ^c	5.92±0.030 ^b	5.74±0.17 ^a
60 days	7.08±0.011 ^d	6.51±0.019 ^d	6.31±0.88 ^c	6.12±0.11 ^c	5.88±0.030 ^b	5.55±0.10 ^a
75 days	7.08±0.078 ^e	6.31±0.085 ^d	6.10±0.085 ^d	5.88±0.10 ^c	5.13±0.16 ^b	4.72±0.016 ^a
90 days	7.08±0.049 ^e	6.25±0.049 ^d	5.84±0.18 ^d	5.15±0.17 ^c	4.68±0.12 ^b	4.15±0.11 ^a

Data in tables 3,4,5 and 6 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

Table 5: Deterioration of total alkaloids content (mg/100mg) in market root of *Solanum indicum* at different relative humidities

Incubation days	Control	30%	50%	75%	96%	100%
1 day	12.20±1.41	12.20±1.41	12.20±1.41	12.20±1.41	12.20±1.41	12.20±1.41
15 days	12.20±1.31 ^c	12.20±1.51 ^c	12.20±1.56 ^a	12.20±1.20 ^a	12.20±1.58 ^a	12.18±1.59 ^a
30 days	12.20±1.30 ^d	12.19±1.30 ^c	12.15±1.51 ^{ab}	12.063±1.46 ^{ab}	11.95±1.20 ^a	11.82±0.17 ^a
45 days	12.20±1.31 ^d	12.10±1.60 ^c	11.99±1.55 ^b	11.94±1.45 ^b	11.80±1.47 ^a	11.71±1.49 ^a
60 days	12.18±1.35 ^d	12.1±1.58 ^c	11.89±1.55 ^b	11.78±1.50 ^b	11.66±1.69 ^a	11.61±1.50 ^a
75 days	12.16±1.36 ^c	11.95±1.54 ^c	11.73±1.49 ^b	11.54±1.42 ^b	11.48±1.39 ^a	11.42±1.40 ^a
90 days	12.15±1.36 ^c	11.86±1.56 ^c	11.65±1.50 ^b	11.48±1.39 ^b	11.42±1.41 ^a	11.31±1.37 ^a

Data in tables 3,4,5 and 6 are the mean of three replicates \pm 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

Table 6: Deterioration of total glycosides content (mg/100 mg) in market root of *Solanum indicum* at different relative humidities

Incubation days	Control	30%	50%	75%	96%	100%
1 day	9.75 \pm 0.051	9.75 \pm 0.051	9.75 \pm 0.051	9.75 \pm 0.051	9.75 \pm 0.051	9.75 \pm 0.051
15 days	9.75 \pm 0.046 ^c	9.75 \pm 0.07 ^c	9.75 \pm 0.070 ^c	9.69 \pm 0.16 ^a	9.50 \pm 0.18 ^a	9.19 \pm 0.15 ^a
30 days	9.75 \pm 0.036 ^d	9.53 \pm 0.61 ^c	9.41 \pm 0.051 ^b	9.36 \pm 0.12 ^b	9.23 \pm 0.047 ^a	9.16 \pm 0.01 ^a
45 days	9.73 \pm 0.026 ^c	9.28 \pm 0.03 ^c	9.12 \pm 0.045 ^{bc}	8.95 \pm 0.051 ^b	8.95 \pm 0.05 ^a	8.84 \pm 0.049 ^a
60 days	9.71 \pm 0.11 ^d	9.1 \pm 0.17 ^c	8.86 \pm 0.041 ^c	8.84 \pm 0.046 ^{ab}	8.69 \pm 0.12 ^a	8.64 \pm 0.15 ^a
75 days	9.67 \pm 0.037 ^c	8.82 \pm 0.072 ^c	8.76 \pm 0.066 ^c	8.83 \pm 0.30 ^{ab}	8.64 \pm 0.073 ^a	8.43 \pm 0.20 ^a
90 days	9.61 \pm 0.53 ^d	8.29 \pm 0.011 ^c	8.086 \pm 0.075 ^b	8.04 \pm 0.06 ^{ab}	7.81 \pm 0.085 ^a	7.73 \pm 0.11 ^a

Data in tables 3,4,5 and 6 are the mean of three replicates \pm 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

Data analysis revealed that reduction of sugars, proteins, phenols, alkaloids and glycosides amount are significantly under influence of relative humidity and incubation days, and it was significant at 5 % level of significance (P value <0.05).

CONCLUSION

Results of this investigation revealed that there were gradual depletion in chemical constituents concentration under different relative humidity which might be due to its degradation into simple form by microbial enzymes and utilization of them by fungi and also it is concluded that lower RH (30 and 50% RH) and short incubation period of drugs have less effectiveness on chemical constituents, this is may be due to less growth of fungi in these unfavorable conditions.

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REFERENCES

1. Nadkarni AK. Indian Materia Medica. 3th edition Dhootapapeshwar, Prakashan; 1951. 1319.
2. Kiritikar KR, Basu BD. Indian Medicinal plants 2nd edition. Vol1. Published by L. M. Basu, Allahabad;1984.
3. Joy PP, Thomas J, Mathew S, Skaria BP. Medicinal Plants. Kerala Agricultural University. 1998; 2:32-35.
4. International seed testing association, International rules for seed health testing, Proc. International seed test assoc; 31, 1966. 1.
5. Roy AK, Chourasia HK, Kumari N. Association of mycoflora with some crude herbal drug of Bhutan. Indian Bot. Repr. 1987; 6(1): 48.
6. Barrnet HL, Hunter BB. Illustrated Genera of Imperfect Fungi. Minneapolis Burgess Publishing Company. Minneapolis; 1972.
7. Thom C, Raper KB. A manual of Aspergillus. Williams and Wilkins. Battimore; 1945.
8. Booth C. The Genus Fusarium. CMI. Kew Surry. U.K; 1971. 237p
9. Nelson PE, Tossoum TA & Marasas WFO. Fusarium Species. An Illustrated Manual for Identification . The Pennsylvania State University Press. U. S. A; 1983. 193.
10. Raper KB & Thomas C. A manual of Penicillia Williams and Wilkins. Battimore; 1949. 850.
11. Lowry OH, Rosebrough NJ, Farr AL & Randall K. Protein measurement with folin phenol reagent. J. Biol. Chemistry. 1951;193:256 – 275.
12. Singh M, Singh SS & Sanwal GG. A new calorimetric method for the determination of

phenolics. *Ind. Phytopath.* 1978; 16: 712 - 714.

- 13. Harborne JB. *Phytochemical methods* - London. New York. Chapman & Hall; 1973. 39-42.
- 14. Kokate CK, Gokhale SB & Purohit AP. *Pharmacognosy Nirali Prakashan Pune*; 2002.
- 15. Sadasivam S & Manickam A. *Biochemical Methods of Agricultural Sciences*. Wiley Estern Ltd. New Delhi. India; 1992.
- 16. Christensen CM, Kaufman HH. *Microflora in Storage of Cereal Grain and Their Products*. C. M. Christensen (Editor). American Association of Cereal Chemists, Inc., St. Paul, MN; 1974.
- 17. Bilgarmi KS, Sinha RK, Prasad T. Effect of fungal flora on the seed contents of moong. *Indian Phytopatho.* 1978; 31: 476-479.
- 18. Kabnoorkar PS, Deokule SS. Studies on changes in chemical constituents roots of Boerheavia diffusa infested with Aspergillus parasiticus and A. niger under storage. *Geobios.* 2009; 36: 206-210.
- 19. Deokule SS, Kabnoorkar PS. Studies on phytochemical constituents of drug Punarnava under storage condition due to fungi. *J. I. B. S.* 2008; 87(3&4): 256 - 261.
- 20. Dutta GR, Roy AK. Mycoflora associated with Strychnous seed deterioration of their active principles under storage, *Indian Phytopath.* 1987; 40 (4): 520.