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## CHANGES IN CARBOHYDRATES AMOUNTS IN FRESH AND MARKET ROOTS OF DRUG *DESMODIUM GANGETICUM* DC. DUE TO FUNGAL FLORA UNDER INFLUENCE OF RELATIVE

M. Rashidi<sup>1\*</sup>, S. S. Deokule<sup>2</sup>

<sup>1</sup>Department of Botany, University of Pune, Pune-411007

### ABSTRACT

The roots of drug *Desmodium gangeticum* DC. were selected for this present investigation. Associated fungi isolated and identified according standard references. In the present study, total 17 fungi were associated with fresh samples and 15 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. Quantitative estimation of carbohydrate relation to association of fungi was done. Maximum growth and percentage incidence of fungi were observed under high relative humidities 75, 96 and 100% RH and long storage periods 45 and 60 days.

**KEYWORDS :** *fungi, deterioration, carbohydrates, relative humidity*

### INTRODUCTION

In Sanskrit, *Desmodium gangeticum* called "Shalaparni". It is commonly called as "Sarivan" or "Darh" (Nadkarni, 1954). Names are from the Gree "desmos", "bond" or "chain" and "hode" like, in reference to the resemblance of the jointed seed pods to links of a chain (Allen and Allen, 1981). *D. gangeticum* belongs to the family of Leguminosae sub family Fabaceae. It is bitter tonic, febrifuge, digestive, anti catarrhal and tonic (Nadkarni, 1954). Whole plant is used for bronchitis, fever and as a tonic unspecified parts are used for dysentery and eye infections (Jain, 1991). The root of *D. gangeticum* is one of the ingredients of a famous Ayurvedic preparation Dashmoola, which is

### Correspondence to Author



M. Rashidi

Department of Botany, University of Pune, Pune-411007

**Email:** Rashidi\_129@yahoo.com

considered to be antipyretic alternative, a bitter tonic and reported to be beneficial in the treatment of typhoid biliousness and also as a diuretic and aphrodisiac. They used for asthma, diarrhoea, cough, dysentery, fever, snake bite antidote and vomiting (Jain, 1991). The presence of contamination in medicinal plant is a serious problem that has recently raised concern. Practices used in harvesting, handling, storage, production and distribution make medicinal plants subject to contamination by various fungi, which may be responsible for spoilage and production of mycotoxins. However, there is scanty information regarding the deterioration of sugar contents of medicinal plants. Therefore this investigation

focused on study associated mycoflora with drug roots of shalparni under storage of different relative humidity (RH) and different incubation days, and other part of this study observed the changes in total sugar (TS) and reducing sugar (RS) due to spoilage of fungi.

## MATERIALS AND METHODS

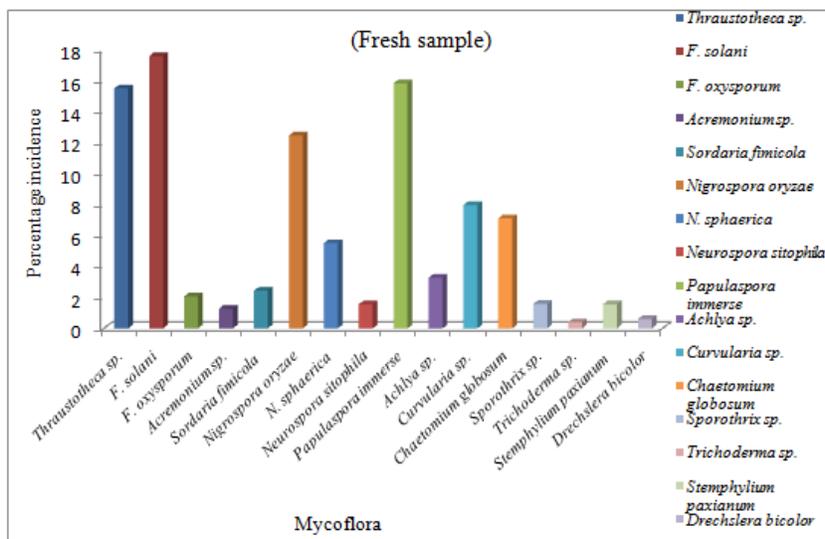
The fresh root samples of Bael were collected from different places of Dapoli, in and around Pune, India. The samples were brought to the laboratory in polyethylene bags to avoid aerial contamination. Blotter test and agar plate method were done for isolation of mycoflora associated with roots. For isolation of internally fungi the roots sterilized with 2% NaOCl solution for some minutes and thoroughly washed with sterilized distilled water. For evaluation of changes in biochemical constituents related to mycoflora, the root samples were stored in small muslin clothes at 30, 50, 75, 96 and 100 % RH at  $28\pm 3^{\circ}\text{C}$  for 90 days. The root samples an internal 15 days were taken out and thoroughly washed with distilled water and plated in Petri plates. The isolation of mycoflora was recorded from first day to 60<sup>th</sup> day of storage. Fungi were identified by using references such as Raper and Thom (1949), Thom and Raper (1945), Barnet and Hunter (1972) and Nelson *et al.* (1983). Some parts of washed root samples were dried in oven and powdered with grinder and were used for biochemical's analysis. Anthrone methods for total carbohydrates and Dinitrosalicilic acid (DNSA) method for reducing

sugar amount (Sadasivam and Manickam, 1992) were followed for biochemical analysis. 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.

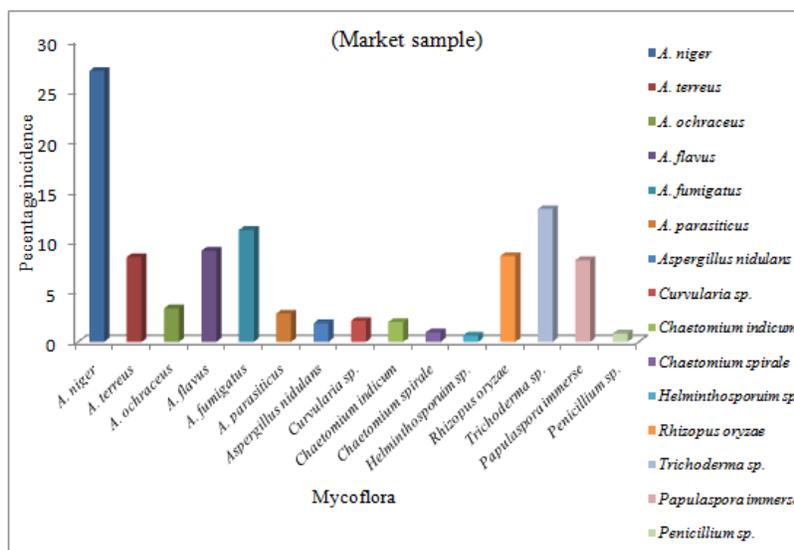
## RESULTS

Total 18 fungi were isolated from the fresh roots of drug *Desmodium gangeticum*; *Fusarium solani* found in the highest percentage 17.56 %, as compared to *Papulaspora immerse* 15.81%, *Thraustotheca* sp.15.47%, *Nigrospora oryzae* 12.44%, *Curvularia lunata*. 7.95%, *Chaetomium globosum* 7.1%, *N. sphaericha* 5.5%, *Achlya* sp. 3.27%, *Sordaria fimicola* 2.43%, *Fusarium oxysporum* 2.07%; while *Neurospora sitophila* and *Sporothrix* sp. in the same percentage 1.58 %, *Acremonium* sp. 1.27% and *Drechslera bicolor* 0.61% observed (Graph 1). Isolated fungi from roots of Shalaparni, which collected from shopkeepers included: *A. niger* found in highest percentage incidence 26.97% after that *Trichoderma* sp. 13.22%, *A. fumigatus* 11.14%, *A. flavus* 9.062%, *Rhizopus oryzae* 8.54%, *A. terreus*, 8.43%, *Papulaspora immerse* 8.12%, *A. ochraceus* 3.33%, *A. parasiticus* 2.81%, *Curvularia lunata* 2.083%, *Chaetomium indicum* 1.97%, and *Aspergillus nidulans* 1.85%. The less percentage incidence of fungi occurred in *Chaetomium spirale*, *Penicillium* sp. and *Helminthosporium* sp. with 0.93, 0.83 and 0.62 % incidences (Graph 2).

Graph 1: Percentage incidence of mycoflora associated with the root of *Desmodium gangeticum* (Fresh sample)



Graph 2: Percentage incidence of mycoflora associated with the root of *Desmodium gangeticum* ( market sample)



The fresh roots of *Desmodium gangeticum*, stored at various relative humidities 30, 50, 75, 96 and 100% RH. Total percentage incidence of fungi under 30% RH and after 15 days were 0.4%, which gradually increased to 1.30% on 60<sup>th</sup> days of incubation. In case of 50, 75, 96 and 100% RH, after 15 days, total percentage incidence of mycoflora observed 0.54, 0.65, 2.72 and 5.03 % and then increased up to 4.47, 7.41, 12.43 and 16.04% after 60 days of storage (Table1). From market samples, 15 fungi were isolated in various percentage incidences under different relative humidities. The total percentage incidence of fungi after 15 days under 30, 50, 75, 96 and 100% RH observed 0.7, 0.91, 1.43, 2.35 and 4.02% which gradually

increased to 2.15, 4.43, 7.01, 11.48 and 16.87% after 60 days of incubation (Table 2).

Fresh roots of *Desmodium gangeticum* contained 37.45% TS and 15.88% RS. In case of 30 and 50 % RH deterioration of sugar contents after 15 days observed 37.37, 15.83% and 36.89, 15.74% while after 90 days of incubation observe these amounts decreased to 33.75, 14.11 and 32.57, 13.48% respectively. More deterioration showed in cases of 75, 96 and 100% RH, after 15 days TS and RS showed 36.53, 15.6%; 35.77, 15.52% and 35.35, 15.38 %, after 90 days of incubation period it showed significant deterioration in each relative humidity 31.06, 13.12%; 30.42, 12.21% and 29.41, 11.44% (Table3). In case of market sample,

deterioration of sugars was more, while after 15 days of storage the value of TS and RS showed 34.51, 15.24%; 34.13, 15.15%; 33.71, 14.84%; 33.12, 14.52% and 32.49, 14.38% under 30, 50, 75, 96 and 100% RH. After 90 days of incubation period these amounts of sugar decreased to 30.76, 13.57%; 29.37, 11.81%, 28.74, 10.85; 27.56, 10.40

and 26.76, 9.95% under different relative humidity, respectively (Table 4).

Analysis of variance showed the effect of relative humidity and incubation days in reduction of total sugar and reducing sugar contents were significant at 5 % level of significance (P value <0.05).

**Table1:** Percentage incidence of fungal isolated from the root of *Desmodium gangeticum* (Fresh sample) 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>Thraustotheca sp.</i>	0.1 0	0.1 0	0.1 0	0.3 2	0.3 2	0.1 0	0.1 0	0.1 0	0.7 6	0.1 0	0.5 4	1.0 9	1.3 0	0.3 2	0.7 6	1.6 3	1.96	0.5 4	1.0 9	1.8 5	2.2 9
<i>F. solani</i>	0.1 0	0.1 0	0.1 0	0.3 2	0.3 2	0.1 0	0.8 7	0.9 8	1.0 9	-	0.3 2	0.9 8	1.1 9	0.8 7	0.8 7	1.3 0	1.85	1.0 9	1.5 2	1.6 3	1.9 6
<i>F. oxysporum</i>	-	-	-	-	0.1 0	-	-	0.1 0	0.1 0	-	0.1 0	0.1 0	0.2 1	0.1 0	-	0.1 0	0.21	0.1 0	0.1 0	0.3 2	0.4 3
<i>Acremonium sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2 1	0.43	-	0.1 0	0.2 1	0.3 2
<i>Sordaria fimicola</i>	-	-	-	-	-	-	-	-	-	-	0.1 0	0.1 0	0.3 2	-	0.1 0	0.1 0	0.43	0.1 0	0.2 1	0.2 1	0.7 6
<i>Nigrospora oryzae</i>	-	-	-	0.1 0	0.1 0	0.1 0	0.1 0	0.5 4	0.6 5	0.1 0	0.1 0	0.9 8	1.0 9	0.2 1	0.3 2	1.0 9	1.63	0.5 4	0.7 6	1.8 5	2.1 8
<i>N. sphaericha</i>	-	-	-	-	-	-	-	-	-	-	-	0.1 0	0.2 1	0.1 0	0.2 1	0.8 7	0.98	0.2 1	0.4 3	0.8 7	1.5 2
<i>Neurospora sitophila</i>	-	-	-	-	-	-	-	-	0.1 0	-	-	0.1 0	0.2 1	-	0.1 0	0.1 0	0.21	0.1 0	0.1 0	0.2 1	0.3 2
<i>Papulaspora immerse</i>	0.1 0	0.1 0	-	0.2 1	0.2 1	0.1 0	0.2 1	0.8 7	0.9 8	0.3 2	0.3 2	0.9 8	1.1 9	0.7 6	0.9 8	1.0 9	1.85	1.0 9	1.1 9	1.3 0	1.9 6
<i>Achlya sp.</i>	-	-	-	-	0.1 0	-	-	0.1 0	0.2 1	-	0.1 0	0.1 0	0.2 1	0.1 0	0.1 0	0.2 1	0.32	0.3 2	0.4 3	0.4 3	0.5 4
<i>Curvularia lunata</i>	-	0.1 0	-	0.1 0	-	0.1 0	0.1 0	0.2 1	0.3 2	0.1 0	0.2 1	0.3 2	0.3 2	0.1 0	0.7 6	0.5 4	0.76	0.5 4	0.9 8	1.0 9	1.3 0
<i>Chaetomium globosum</i>	-	-	-	-	-	-	-	-	-	-	-	0.1 0	0.1 0	-	-	0.2 1	0.32	0.1 0	0.1 0	0.3 2	0.3 2
<i>Sporothrix sp.</i>	-	-	-	0.1 0	0.1 0	-	-	0.2 1	0.2 1	-	0.1 0	0.5 4	0.5 4	0.1 0	0.3 2	0.9 8	0.98	0.1 0	0.4 3	1.0 9	1.3 0
<i>Trichoderma sp.</i>	-	-	-	-	-	-	-	-	-	-	-	0.2 1	0.1 0	-	-	0.3 2	-	0.1 0	0.1 0	0.4 3	0.3 2
<i>Stemphylium paxianum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.10	-	0.1 0	0.1 0	0.1 0
<i>Drechslera bicolor</i>	-	-	-	-	-	-	-	-	-	-	-	0.1 0	0.3 2	-	0.1 0	0.2 1	0.21	0.1 0	0.1 0	0.2 1	0.2 1
<i>Theilavia terricola</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1 0	0.10	-	0.1 0	0.1 0	0.2 1
Total	0.3 0	0.4 0	0.2 0	1.1 9	1.3 0	0.5 4	1.4 1	3.1 6	4.4 7	0.6 5	1.9 6	5.8 8	7.4 1	2.7 2	5.1 2	9.1 6	12.4 3	5.0 3	7.8 4	12. 22	16. 04

**Table2:** Percentage incidence of fungal isolated from the root of *Desmodium gangeticum* (Market sample) 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>	0.2 0	0.2 0	0.3 1	0.4 1	0.5 2	0.3 1	0.5 2	0.9 3	1.1 4	0.8 3	0.9 3	1.1 4	1.5 6	1.0 41	1.5 6	2.1 8	2.9 1	1.5 6	1.9 7	2.9 1	3.7 0
<i>A. fumigatus</i>	0.1 0	0.1 0	0.1 0	0.2 0	0.1 0	0.2 0	0.3 1	0.4 1	0.1 0	0.3 1	0.5 2	0.7 2	0.2 0	0.5 2	0.9 3	1.5 6	0.3 1	0.9 3	1.5 6	1.7 7	
<i>A. flavus</i>	0.1 0	0.1 0	0.1 0	0.2 0	0.3 1	0.1 0	0.2 0	0.4 1	0.5 2	0.1 0	0.4 1	0.5 2	0.8 3	0.2 0	0.4 1	0.5 2	0.9 3	0.3 1	0.5 2	0.9 3	1.2 5
<i>A. terreus</i>	-	-	-	0.1 0	0.2 0	-	-	0.2 0	0.4 1	0.1 0	0.5 2	0.7 3	0.9 3	0.2 0	0.5 2	0.7 3	0.8 3	0.3 1	0.5 2	0.9 3	1.1 4
<i>A. ochraceus</i>	-	-	-	-	-	-	-	0.2 0	-	0.1 0	0.1 0	0.2 0	0.1 0	0.1 0	0.2 0	0.4 1	0.2 0	0.4 1	0.5 2	0.7 2	
<i>A. parasiticus</i>	-	-	-	-	-	-	-	-	-	-	-	0.2 0	-	-	0.1 0	0.4 1	0.1 0	0.3 1	0.7 2	0.9 3	
<i>Aspergillus sp.</i>	-	-	-	-	-	-	-	-	-	-	-	0.1 0	-	-	0.1 0	0.2 0	0.1 0	0.2 0	0.6 2	0.5 2	
<i>Trichoderma sp.</i>	0.2 0	0.1 0	0.2 0	0.3 1	0.4 1	0.2 0	0.5 2	0.8 3	0.9 3	0.1 0	0.2 0	0.6 3	0.9 3	0.3 1	0.7 3	0.9 6	1.5 1	0.4 2	0.7 3	1.1 4	1.7 7
<i>Rhizopus oryzae</i>	0.1 0	0.1 0	0.1 0	0.2 0	0.1 0	0.2 0	0.3 1	0.4 1	0.1 0	0.2 0	0.3 1	0.5 2	0.2 0	0.3 1	0.7 2	0.8 3	0.4 1	0.7 2	1.1 4	1.3 5	
<i>Papulaspora immerse</i>	0.1 0	0.1 0	0.1 0	0.2 0	0.3 1	0.1 0	0.2 0	0.3 1	0.4 1	0.1 0	0.1 0	0.3 1	0.7 2	0.1 0	0.2 0	0.5 2	0.9 2	0.3 1	0.5 2	0.9 2	1.4 5
<i>Curvularia lunata</i>									-	0.1 0	0.2 0	-	-	0.1 0	0.2 0	0.3 1	-	-	0.4 1	0.7 2	
<i>Chaetomium indicum</i>										-	0.1 0	0.1 0	-	-	0.2 1	0.3 1	-	0.3 1	0.4 1	0.5 2	
<i>Ch. Spirale</i>										-	0.1 0	0.1 0	-	-	-	0.1 0	-	-	0.2 1	0.4 1	
<i>Helminthosporium sp.</i>															-	0.1 0	-	-	0.2 0	0.3 1	
<i>Penicillium sp.</i>												-	0.1 0	-	-	0.1 0	0.1 0	-	-	0.2 0	0.3 1
Total	0.8	0.7	0.9 1	1.4 2	2.1 5	0.9 1	1.8 4	3.3 3.3	4.4 3	1.4 3	2.8 7	4.6 4	7.0 1	2.3 51	7.4 3	7.4 3	11. 48	4.0 2	7.1 3	12. 82	16. 87

**Table3:** Deterioration of Total sugars (TS) and Reducing sugars (RS) content (mg/100mg) in root of *Desmodium gangeticum* (Fresh sample) 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	37.45 ±0.19	15.88 ±0.045	37.45 ±0.19	15.88 ±0.045	37.45 ±0.19	15.88 ±0.045	37.45 ±0.19	15.88 ±0.045	37.45 ±0.19	15.88 ±0.045	37.45 ±0.19	15.88 ±0.045
15 days	37.45 ±0.072 <sup>c</sup>	15.88 ±0.11 <sup>c</sup>	37.37 ±0.12 <sup>c</sup>	15.83 ±0.11 <sup>c</sup>	36.89 ±0.12 <sup>c</sup>	15.74 ±0.045 <sup>c</sup>	36.53 ±0.26 <sup>b</sup>	15.6 ±0.045 <sup>b</sup>	35.77 ±0.31 <sup>b</sup>	15.52 ±0.026 <sup>b</sup>	35.35 ±0.12 <sup>a</sup>	15.38 ±0.11 <sup>a</sup>
30 days	37.45 ±0.14 <sup>c</sup>	15.88 ±0.094 <sup>c</sup>	37.03 ±0.31 <sup>c</sup>	15.70 ±0.094 <sup>c</sup>	36.44 ±0.29 <sup>c</sup>	15.47 ±0.11 <sup>c</sup>	35.52 ±0.19 <sup>b</sup>	15.38 ±0.094 <sup>b</sup>	34.80 ±0.31 <sup>b</sup>	15.11 ±0.11 <sup>b</sup>	34.1 ±0.072 <sup>a</sup>	14.93 ±0.026 <sup>a</sup>
45 days	37.45 ±0.072 <sup>c</sup>	15.88 ±0.011 <sup>c</sup>	36.48 ±0.21 <sup>c</sup>	15.42 ±0.24 <sup>c</sup>	35.35 ±0.12 <sup>c</sup>	15.20 ±0.17 <sup>c</sup>	34.25 ±0.19 <sup>b</sup>	14.93 ±0.094 <sup>b</sup>	33.29 ±0.69 <sup>ab</sup>	14.47 ±0.069 <sup>ab</sup>	32.23 ±0.47 <sup>a</sup>	14.25 ±0.069 <sup>a</sup>
60 days	37.45 ±0.26 <sup>c</sup>	15.88 ±0.013 <sup>c</sup>	35.73 ±0.37 <sup>c</sup>	15.24 ±0.23 <sup>c</sup>	34.51 ±0.62 <sup>c</sup>	14.93 ±0.069 <sup>c</sup>	33.08 ±0.33 <sup>b</sup>	14.66 ±0.11 <sup>b</sup>	31.98 ±0.44 <sup>ab</sup>	14.25 ±0.094 <sup>a</sup>	31.86 ±0.26 <sup>a</sup>	14.16 ±0.045 <sup>a</sup>
75 days	37.45 ±0.19 <sup>c</sup>	15.88 ±0.015 <sup>c</sup>	34.59 ±0.19 <sup>c</sup>	14.70 ±0.29 <sup>c</sup>	34.006 ±0.14 <sup>c</sup>	14.43 ±0.069 <sup>c</sup>	31.81 ±0.88 <sup>b</sup>	14.02 ±0.40 <sup>b</sup>	29.41 ±0.55 <sup>ab</sup>	13.12 ±0.20 <sup>ab</sup>	27.48 ±0.31 <sup>a</sup>	12.48 ±0.22 <sup>a</sup>
90 days	46.29 ±0.072 <sup>c</sup>	15.88 ±0.11 <sup>c</sup>	33.75 ±0.56 <sup>c</sup>	14.11 ±0.14 <sup>c</sup>	32.57 ±0.55 <sup>c</sup>	13.48 ±0.094 <sup>c</sup>	31.06 ±0.57 <sup>b</sup>	13.12 ±0.14 <sup>b</sup>	30.42 ±0.21 <sup>a</sup>	12.21 ±0.45 <sup>a</sup>	29.41 ±0.55 <sup>a</sup>	11.44 ±0.23 <sup>a</sup>

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

## DISCUSSION

These fungi at first invade external surface which latter invade to deeply parts of plant drugs under suitable condition especially good level of moisture contents. These fungi consume nutrients in tissue plants by slow break down of constituents in tissues. Relative humidity is most effective factor on deterioration and loss of constituents in stored herbal drugs due to naturally growing fungi and other microorganisms. Fungi divided into two ecological categories such as field and storage fungi. Field fungi observe to invade developing or mature tissues of plants. The major field fungi genera are *Alternaria*, *Helminthosporium*, *Fusarium* and *Cladosporium*. Storage fungi are those encountered on plants at moisture condition routinely found in stored products, *Aspergillus* and *Penicillium* are principally species (Aziz *et al.* 1998). Carbohydrates have been shown to play an important role during plant-pathogen interactions. Carbohydrates are the basic building blocks for the synthesis of various defense chemicals such as phenolics, phytoalexins and lignin. Hence the quality and quantity of sugars play an important role in diseases resistance (Tharanathan *et al.* 1987). Fungal activity can cause changes during storage of herbal plants and their products that are detrimental to nutritive value. Specifically, nutrients are lost because of changes in carbohydrates, protein, phenols and other constituents. Conditions that favour for fungal activity lead to carbohydrate decomposition. Sugars are consumed and converted into CO<sub>2</sub> and H<sub>2</sub>O. The drugs stored at higher relative humidity (75, 96 and 100%RH) favoured for maximum deterioration of sugar for naturally growing fungi on the drugs. Reduction in sugar amount of herbal drugs associated with fungi under storage is accordance with result of some earlier authors such as; Kumar and Nair, 1981; Prasad and Das, 1985; Ghosh *et al.* 1981; Lunch *et al.* 1962; Kabnoorkar and Deokule, 2009; Rashidi *et al.* 2012 and Mahadshwari *et al.* 1984.

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