



# Biodeterioration of carbohydrates in genuine and market roots of drug *Oroxylum indicum* Vent. under storage due to spoilage of fungi

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## ABSTRACT

The fresh and market roots of drug *Oroxylum indicum* Vent. were selected for the present investigation. Total of 14 fungi were associated with fresh samples and 13 fungi were isolated from market samples. In the fresh samples, *Trichoderma* sp. found in high percentage incidence followed by *Fusarium solani*, while, *Chaetomium indicum* and *Cunninghamella elegans* observed in very less percentage incidence and in market samples *Aspergillus niger* had highest percentage incidence and *Drechslera bicolor* occurred in less percentage incidence. 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 estimated. Maximum growth and percentage of incidence of fungi and also biodeterioration of carbohydrates were observed under high relative humidities 75, 96 and 100% RH and with the length of incubation days 45 and 60 days.

Key words: Fungi, deterioration, carbohydrates, relative humidity.

## Introduction

*Oroxylum indicum* is commonly called as "Shivnak", "Arlu", and "Tetu" or "mid night horror". This plant is a member of the "Bignoniaceae" family. The root barks are an ingredient of the Dashmoola of Hindu Medicine. The root barks are an ingredient of the Dashmoola of Hindu Medicine. The root-bark contains a crystalline bitter glycoside substance named "Oroxylon" or "Oroxylin" in addition to an acrid principle, pectin, extractive matter, crystalline fat, wax, chlorophyll astringent principle and critic acid (Nadkarni, 1954). The root bark is useful in diarrhea and dysentery. The roots are used in dropsy and as vulnerary and leaves are reputed as emollient. Roots are used for the treatment of tuberculosis (Bhattacharje, 2000). The root bark is used in fever, bronchitis, intestinal worms, leucoderma asthma, inflammation and troubles (Kritikar and Basu, 1984). Traditional systems of harvesting, handling, storage, production and distribution of medicinal plants make them subject to contamination by

various microorganism. However, there is scanty information regarding the associated mycoflora with herbal drugs in fresh and under storage and also deterioration of carbohydrates contents in herbal drugs. Therefore the present investigation designed to study associated mycoflora with the fresh and market root drug of *O. indicum* under storage of different relative humidity (RH) and different incubation days, and other part of this study studied the changes in total sugar (TS) and reducing sugar (RS) due to spoilage of isolated fungi and compared changes in carbohydrate amounts in fresh and market samples.

## Material and Methods

The fresh roots of drug *Oroxylum indicum* were collected in healthy, flowering and fruiting conditions from different localities in Maharashtra, India. Market



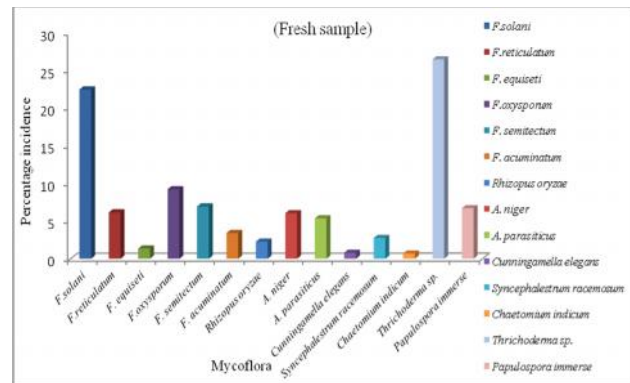
drug samples were also collected from various Shopkeepers and Kashthaushadhi. Samples were brought to the laboratory in polyethylene bags and were cut into small pieces and soaked for 2 minutes in 2 sodium hypochlorite solution then thoroughly washed with sterilized distilled water. Ten pieces of roots 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 of 15 days, the root samples were taken out and thoroughly washed with distilled water and plated in Petri plates and they were incubated at 25°C and after 3-4 days, developed colonies of fungi observed and the percentage incidence of mycoflora was recorded. Different fungi were identified at genera and species level by using references, such as Raper and Thom (1949), Thom and Raper (1945), Barnet and Hunter (1972), Booth (1971) Nelson et al. (1983) and Ellis (1971). Some parts of washed root samples were dried in oven and powdered with grinder and were used for biochemical analysis. Anthrone methods for total carbohydrates and Dinitrosalicilic acid (DNSA) method for reducing sugar content (Sadasivam and Manickam, 1992) were estimated. Simple correlation was run between selected parameters using Statistical Package for Social Science (SPSS) software in which statistical significance was determined at 0.05 %probability levels.

## Results

### Mycoflora associated with fresh and market samples

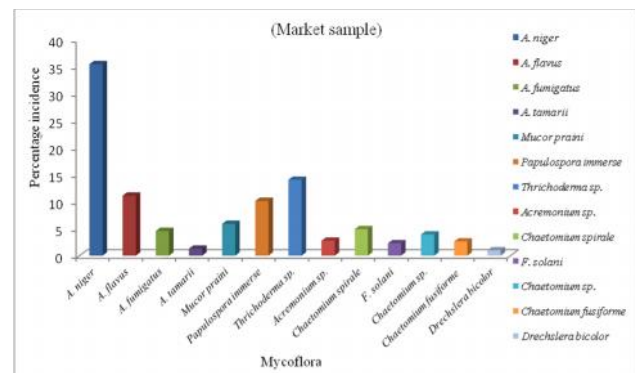
Total of 14 fungi were isolated from fresh roots of Oroxylum indicum, Fusarium solani, F. reticulatum, F. equiseti, F oxysporum, F. semitectum, F. acuminatum, Rhizopus oryzae, A. niger, A. parasiticus, Cunninghamella elegans, Syncephalestrum racemosum, Chaetomium indicum, Trichoderma sp. and Papulaspora immerse. The percentage incidence of all mentioned fungi are listed such as: Fusarium solani observed 22.52% while F. reticulatum 6.17% F. equiseti 1.73% F. oxysporum 9.25% F. semitectum 6.95% F. acuminatum 3.43, Rhizopus oryzae 2.28, A. niger 6.057, A. parasiticus 5.38% Cunninghamella elegans 0.8, Chaetomium indicum 0.69, Trichoderma sp. 26.52% and Papulaspora immerse 6.73%(Fig. 1).

Fig - 1 Percentage incidence of mycoflora associated with the root o f Oroxylum indicum (Fresh sample)



Total 13 fungi were isolated from market roots of Oroxylum indicum; A. niger 35.45% Trichoderma sp. 14.05% A. flavus, 11.11% Papulaspora immerse, 10.13% Mucor praini 5.88% Chaetomium spirale 4.91% A. fumigatus 4.57% Chaetomium sp. 3.93% Chaetomium fusiforme, 2.62% Acremonium sp. 2.77% F. solani 2.29% A. tamarii 1.3% and Drechslera bicolor, 0.99% (Fig. 2).

Fig - 2 Percentage incidence of mycoflora associated with the root o f Oroxylum indicum (market sample)



### The effect of relative humidity and incubation days on fungal growth

The fresh root sample of Oroxylum indicum stored under different relative humidities and the percentage incidence of mycoflora calculated. Total percentage incidence of fungi under 30 %RH after 15 days of storage period was 0.44 % this percentage of incidence increased to 2.47% after 60 days of storage period. Similarly the % incidence observed in cases of 50, 75 and 96 % RH, after 15 days of incubation were 0.55, 0.99 and 1% and increased up to 4.28, 7.03 and 9.56% after 60 days. Lastly in 100% RH the percentage incidences were 3.83, 7.37, 12.17 and 18.69 % after 15, 30, 45 and 60 days of incubation. In case of 100 % RH and 60 days of

incubation period each fungi showed the following percentage incidence: *F. solani* 3.88, *F. reticulatum* 1.28, *F. equiseti* 0.57, *F. oxysporum* 1.71, *F. semitectum* 1.029, *F. acuminatum* 0.8, *Rhizopus oryzae* 0.34, *Aspergillus niger* 1.71, *Cunninghamella elegans* 0.57, *Syncephalestrum racemosum* 0.8%, *Trichoderma* sp. 3.2%, *Chaetomium indicum* 0.34% and *Papulaspora immerse* 1.25% respectively. Out of these 14 fungi; *F. solani* was observed in high percentage incidence. *Chaetomium indicum* and *Cunninghamella elegans* were very less and they were observed after 45 days of incubation in 100% RH (Table 1).

Market samples of this drug, stored at different relative humidities. Total percentage incidence of fungi under different relative humidity calculated. In the case of 30% RH total percentage incidence observed 0.97, this amount increased to 1.13% in 60 days of incubation. In other cases of relative humidity 50, 75, 96 and 100% RH after 60 days of incubation, observed 1.78, 8.28, 10.4 and 16.62%. In all isolated fungi *Aspergillus niger*, *Trichoderma* sp., *A. flavus*, *A. fumigatus*, *Papulaspora immerse* and *Mucor praini* observed in high percentage incidence after 60 days of incubation under 100% RH, 4.57, 2.45, 1.79 and 1.79 and 1.14 % *Aspergillus fumigatus*, *A. tamarii*, *Acremonium* sp., *Fusarium solani*, *Chaetomium spirale*, *Chaetomium* sp., *A. terricola* and *Drechslera bicolor* showed different percentage incidence under different relative humidity and different incubation days. *Drechslera bicolor* observed in very less percentage, other fungi *A. terricola*, *F. solani* and *A. tamarii* with same percentage incidence 0.49 % on 60<sup>th</sup> days of storage under 100 % RH observed. *A. fumigatus* and *Chaetomium spirale* observed on 60<sup>th</sup> days of incubation under 30% RH (Table 2).

### Changes in carbohydrate in fresh and market sample under storage

Fresh roots of *Oroxylum indicum* stored at different relative humidities 30, 50, 75, 96 and 100% RH. In case of 30 % RH, values of TS and RS, after 15 days observed similar to control; 62.28 and 23.98% but gradually decreased while after 90 days value of sugars showed maximum deterioration 56.01, 13.57%. Similarly, in other cases of RH, value of sugars showed maximum decline after 90 days of storage period, under 50, 75, 96 and 100% RH; 55.42, 10.85% 53.07, 9.50; 51.22, 9.049 and 50.54, 8.14% (Table 3).

Market sample showed more deterioration as compared to fresh sample. After 15 days under all relative humidity 30, 50, 75, 96 and 100 % RH, TS and RS amount observed 50.08, 21.71% 49.36, 19.45% 48.19, 19.004 ; 47.93, 18.06% and 47.09, 16.74% respectively. These values of sugars gradually decreased, while after

90 days of incubation period recorded 42.50, 11.31; 41.16, 10.40% 41.15, 9.04; 40.15, 8.59% and 38.29, 7.69, respectively (Table 4). Data analysis of sugar amounts indicated that there were a significant correlation between incubation days and relative humidity with deterioration of sugar contents at 5% levels of significance (P value <0.05).

### Discussion

There was 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.

Sugars occupy a central position in plant metabolism, not only sugars are the first complex organic compounds formed in the plant as a result of photosynthesis, but also they provide a major source of respiratory energy. In addition many other classes of plant constituents, the nucleic acids and the plant glycosides, contain sugars as essential features of their structures. Finally sugar play a number of ecological roles, in plant- animal interaction (flower nectars), in protection from wounding, infection and in the detoxification of foreign substances (Harborne, 1973). Fungal activity can cause changes during storage of herbal plants and their products that are detrimental to nutritive value. 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 in the present study. Reduction in sugar amount of herbal drugs associated with fungi under storage is accordance with result of some previous records of Kumar and Nair, (1981); Ghosh et al. (1981); Lunch et al. (1962); Kabnoorkar and Deokule, (2009); Rashidi et al. (2012) and Mahadshwari et al. (1984). The result of this investigation indicate that the maximum reduction of carbohydrates was after 60<sup>th</sup> to 90<sup>th</sup> days of incubations while total percentage incidence of fungi were also maximum in mycoflora associated with fresh and market



samples. In part of biochemical analysis of drugs it was observed that relative humidities 75, 96 and 100% RH show the significant reduction in total sugars. More reduction in market samples of all

selected herbal plants as compared to fresh samples was showed. This may be due to unscientific methods of harvesting, collecting, handling and storage in unsuitable places, transporting and drying.

Table - 1 Percentage incidence of fungal isolated from the root of Oroxyllum indicum (Fresh sample) stored at various relative humidity

Mycoflora	Conc.	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	0.22	0.11	0.34	0.8	1.25	0.11	0.57	0.68	1.48	0.22	0.8	1.029	1.94	0.34	0.91	1.25	2.28	0.8	1.029	2.4	3.88
F. reticulatum	-	-	-	-	0.11	-	0.11	0.11	0.22	0.11	0.11	0.22	0.34	0.11	0.22	0.45	0.57	0.34	0.8	1.029	1.25
F. equiseti	-	-	-	-	-	-	-	-	-	-	-	-	0.11	-	-	-	0.11	0.11	0.11	0.34	0.57
F. oxysporum	0.11	-	-	-	0.11	0.11	0.22	0.22	0.57	0.11	0.45	0.57	0.91	-	0.11	0.34	0.8	0.57	1.029	1.25	1.71
F. semitectum	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.22	0.11	0.11	0.22	0.34	0.11	0.34	0.57	0.8	0.22	0.45	0.57	1.029
F. acuminatum	-	-	-	-	0.11	-	-	-	0.11	-	-	0.22	0.34	-	0.11	0.34	0.57	0.11	0.22	0.45	0.8
Rhizopus oryzae	-	-	-	-	-	-	-	0.11	0.22	0.11	-	0.11	0.22	-	0.11	0.22	0.34	0.11	0.11	0.22	0.34
pergillus niger	-	-	-	-	-	-	-	-	0.11	-	-	0.22	0.57	0.11	0.34	0.57	1.029	0.11	0.45	0.8	1.71
Aspergillus parasiticus	-	-	-	-	0.11	-	-	0.11	0.22	-	-	0.11	0.34	0.11	0.22	0.45	0.57	0.22	0.57	1.029	1.25
Cunningamella elegans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.22	0.57
Syncephalestrum racemosum	-	-	-	-	0.11	-	-	-	0.11	-	-	0.11	0.22	-	0.11	0.22	0.34	0.11	0.22	0.34	0.8
Trichoderma sp.	0.11	0.11	0.11	0.22	0.45	0.11	0.34	0.57	0.8	0.22	0.8	1.029	1.25	0.11	0.34	0.91	1.37	1.029	1.71	2.4	3.2
Chaetomium indicum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.11	-	-	0.22	0.34
Papulaspora mmerse	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.22	0.11	0.11	0.34	0.45	0.11	0.22	0.57	0.68	0.11	0.68	0.91	1.25
Total	0.66	0.44	0.67	1.24	2.47	0.55	1.46	2.02	4.28	0.99	2.38	4.178	7.03	1	3.03	5.89	9.569	3.839	7.378	12.17	18.69

Table - 2 Percentage incidence of fungal isolated from the root of Oroxyllum indicum (Market sample) stored at various relative humidity

Mycoflora	Conc.	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
Aspergillus niger	0.65	0.65	0.65	0.81	1.14	0.65	1.30	1.47	2.45	1.30	1.47	2.45	2.77	0.81	1.47	1.79	2.94	1.14	1.79	3.10	4.57
A. flavus	0.16	0.16	0.16	0.32	0.32	0.16	0.16	0.49	0.65	0.16	0.32	0.49	0.81	0.16	0.32	0.65	1.30	0.16	0.81	1.47	1.79
A. fumigatus	-	-	-	-	0.16	-	-	0.16	0.16	-	0.16	0.32	0.49	-	0.16	0.32	0.65	0.16	0.32	0.65	0.81
A. tamarii	-	-	-	-	-	-	-	-	0.16	-	-	-	0.16	-	-	-	0.16	-	-	0.32	0.49
Mucor praini	-	-	-	-	0.16	-	-	-	0.32	-	0.16	0.16	0.49	0.16	0.16	0.32	0.81	0.16	0.81	0.98	1.14
Papulaspora immerse	-	-	-	0.16	0.16	-	0.16	0.32	0.49	0.16	0.32	0.65	0.81	0.16	0.65	0.81	0.98	0.16	0.81	1.47	1.79
Trichoderma sp.	0.16	0.16	0.16	0.16	0.32	0.16	0.16	0.32	0.49	0.16	0.65	0.81	0.98	0.32	0.65	1.30	1.47	0.49	1.14	1.47	2.45
Acremonium sp.	-	-	-	-	-	-	-	-	0.16	-	-	-	0.16	-	-	0.16	0.32	-	0.49	0.65	0.81
Fusarium solani	-	-	-	-	-	-	-	0.16	0.32	-	-	0.16	0.32	-	-	0.16	0.32	-	-	0.32	0.49
Chaetomium spirale	-	-	-	-	0.16	0.16	-	-	0.16	-	-	0.16	0.32	-	0.16	0.32	0.49	0.49	0.65	0.81	0.98
Chaetomium sp.	-	-	-	-	0.16	-	-	0.16	0.16	-	0.16	0.16	0.49	-	0.16	0.32	0.49	0.16	0.32	0.49	0.65
A. terricola	-	-	-	-	0.16	-	-	-	0.16	-	-	0.16	0.32	-	0.16	0.16	0.32	0.16	0.16	0.32	0.49
Drechslera bicolor	-	-	-	-	-	-	-	-	-	-	-	0.16	0.16	-	-	0.16	0.16	-	-	0.16	0.16
Total	0.97	0.97	0.97	1.45	2.74	1.13	1.78	3.08	5.68	1.78	3.24	21.52	8.28	1.61	3.89	6.47	10.4	3.08	6.81	12.21	16.62



Table - 3 Deterioration of total sugars (TS) and reducing sugars (RS) content (mg/100mg) in root of *Oroxylum indicum* (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	62.41 ±0.47	23.98 ±3.26	2.41 ±0.47	23.98 ±3.26	62.41 ±0.47	23.98 ±3.26	62.41 ±0.47	23.98 ±3.26	62.41 ±0.47	23.98 ±3.26	62.41 ±0.47	23.98 ±3.26
15 days	62.41 ±0.47 <sup>c</sup>	23.98 ±3.26 <sup>c</sup>	62.28 ±0.38 <sup>c</sup>	23.98 ±3.51 <sup>c</sup>	61.44 ±0.31 <sup>c</sup>	22.62 ±3.71 <sup>c</sup>	60.69 ±0.14 <sup>b</sup>	21.26 ±3.17 <sup>b</sup>	60.47 ±0.12 <sup>ab</sup>	20.81 ±2.27 <sup>a</sup>	59.89 ±0.62 <sup>a</sup>	19.45 ±2.51 <sup>a</sup>
30 days	62.41 ±0.47 <sup>d</sup>	23.98 ±3.26 <sup>d</sup>	61.36 ±0.45 <sup>cd</sup>	23.52 ±3.42 <sup>cd</sup>	60.14 ±0.44 <sup>c</sup>	18.55 ±3.53 <sup>c</sup>	59.34 ±0.33 <sup>b</sup>	19.004 ±2.35 <sup>b</sup>	58.54 ±0.69 <sup>ab</sup>	18.55 ±1.19 <sup>ab</sup>	57.70 ±0.45 <sup>a</sup>	15.83 ±2.72 <sup>a</sup>
45 days	62.41 ±0.47 <sup>d</sup>	23.98 ±3.26 <sup>d</sup>	60.52 ±0.14 <sup>c</sup>	22.62± 3.27 <sup>c</sup>	59.38 ±0.072 <sup>bc</sup>	16.74 ±2.75 <sup>c</sup>	58.75 ±0.51 <sup>b</sup>	15.83 ±1.35 <sup>b</sup>	56.86 ±0.31 <sup>ab</sup>	15.38 ±1.35 <sup>ab</sup>	55.97 ±0.44 <sup>a</sup>	14.47 ±1.94 <sup>a</sup>
60 days	62.41 ±0.47 <sup>c</sup>	23.98 ±3.26 <sup>c</sup>	59.51 ±0.19 <sup>c</sup>	15.83± 1.97 <sup>c</sup>	58.50 ±0.44 <sup>c</sup>	14.47 ±2.65 <sup>c</sup>	57.57 ±0.57 <sup>b</sup>	14.027 ±2.82 <sup>b</sup>	56.10 ±0.62 <sup>a</sup>	13.57 ±1.82 <sup>a</sup>	54.25 ±0.19 <sup>a</sup>	12.21 ±2.27 <sup>a</sup>
75 days	62.41 ±0.47 <sup>c</sup>	23.98 ±3.26 <sup>c</sup>	57.82 ±0.63 <sup>c</sup>	15.38± 2.073 <sup>c</sup>	57.02 ±1.27 <sup>bc</sup>	12.21 ±2.073 <sup>bc</sup>	54.58 ±0.14 <sup>b</sup>	11.31 ±3.42 <sup>b</sup>	54.58 ±0.14 <sup>ab</sup>	9.95 ±2.65 <sup>a</sup>	53.53 ±0.45 <sup>a</sup>	9.50 ±1.88 <sup>a</sup>
90 days	62.41 ±0.47 <sup>c</sup>	23.98 ±3.26 <sup>c</sup>	56.01 ±0.69 <sup>c</sup>	13.57± 0.90 <sup>c</sup>	55.42 ±1.38 <sup>bc</sup>	10.85 ±2.27 <sup>abc</sup>	53.07 ±0.31 <sup>ab</sup>	9.50 ±3.33 <sup>ab</sup>	51.22 ±0.52 <sup>a</sup>	9.049 ±2.90 <sup>a</sup>	50.54 ±0.07 <sup>a</sup>	8.14 ±0.69 <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

Table - 4 Deterioration of Total sugars (TS) and Reducing sugars (RS) content (mg/100mg) in root of *Oroxylum indicum* (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	50.92 ±0.62	21.71 ±2.76	50.92 ±0.62	21.71 ±2.76	50.92 ±0.62	21.71 ±2.76	50.92 ±0.62	21.71 ±2.76	50.92 ±0.62	21.71 ±2.76	50.92 ±0.62	21.71 ±2.76
15 days	50.92 ±0.62 <sup>d</sup>	21.71 ±2.76 <sup>d</sup>	50.08 ±0.62 <sup>c</sup>	21.71 ±2.57 <sup>c</sup>	49.36 ±0.12 <sup>bc</sup>	19.45 ±2.32 <sup>bc</sup>	48.19 ±0.26 <sup>b</sup>	19.004 ±2.76 <sup>ab</sup>	47.93 ±0.07 <sup>ab</sup>	18.06 ±2.32 <sup>ab</sup>	47.09 ±0.37 <sup>a</sup>	16.74 ±2.39 <sup>a</sup>
30 days	50.92 ±0.62 <sup>c</sup>	21.71 ±2.76 <sup>d</sup>	48.62 ±0.57 <sup>c</sup>	21.26 ±2.82 <sup>c</sup>	48.02 ±0.072 <sup>bc</sup>	20.26 ±2.39 <sup>bc</sup>	46.67 ±0.19 <sup>b</sup>	18.09 ±2.39 <sup>b</sup>	45.91 ±0.69 <sup>a</sup>	17.64 ±2.27 <sup>a</sup>	44.65 ±0.69 <sup>a</sup>	15.83 ±2.32 <sup>a</sup>
45 days	50.92 ±0.62 <sup>c</sup>	21.71 ±2.76 <sup>d</sup>	47.85 ±0.33 <sup>c</sup>	20.36 ±2.90 <sup>c</sup>	46.71 ±0.12 <sup>c</sup>	19.004 ±2.32 <sup>bc</sup>	45.24 ±0.59 <sup>b</sup>	18.09 ±2.26 <sup>ab</sup>	44.27 ±0.14 <sup>ab</sup>	15.83 ±1.82 <sup>ab</sup>	43.18 ±0.33 <sup>a</sup>	14.47 ±1.62 <sup>a</sup>
60 days	50.92 ±0.62 <sup>c</sup>	21.71 ±2.76 <sup>c</sup>	47.13 ±0.95 <sup>c</sup>	19.004 ±1.97 <sup>c</sup>	45.37 ±0.26 <sup>c</sup>	17.64 ±2.13 <sup>bc</sup>	44.19 ±0.25 <sup>b</sup>	15.38 ±2.08 <sup>ab</sup>	43.09 ±0.19 <sup>ab</sup>	14.02 ±0.26 <sup>ab</sup>	41.75 ±1.27 <sup>a</sup>	12.68 ±0.69 <sup>a</sup>
75 days	50.92 ±0.62 <sup>c</sup>	21.71 ±2.76 <sup>c</sup>	44.73 ±0.51 <sup>c</sup>	14.47 ±2.39 <sup>c</sup>	43.26 ±0.51 <sup>bc</sup>	14.027 ±2.49 <sup>bc</sup>	41.16 ±0.47 <sup>ab</sup>	13.57 ±4.08 <sup>ab</sup>	40.19 ±0.66 <sup>a</sup>	10.85 ±1.58 <sup>a</sup>	40.12 ±0.19 <sup>a</sup>	9.04± 0.69 <sup>a</sup>
90 days	50.92 ±0.62 <sup>c</sup>	21.71 ±2.76 <sup>c</sup>	42.50 ±0.12 <sup>c</sup>	11.31 ±1.38 <sup>c</sup>	41.16 ±0.52 <sup>abc</sup>	10.40 ±2.04 <sup>bc</sup>	41.15 ±0.12 <sup>ab</sup>	9.04 ±2.94 <sup>ab</sup>	40.15 ±0.12 <sup>a</sup>	8.59 ±2.23 <sup>a</sup>	38.29 ±0.62 <sup>a</sup>	7.69 ±1.71 <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.

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