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(Research Article)

Fungal Association and Deterioration of Chemical Constituents of *Oroxylum indicum* (Vent.) Roots Under the Influence of Different Relative Humidity

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ABSTRACT

In the present study, total 14 fungal species were found to be associated with Oroxylum indicum Vent. roots such as, Fusarium solani, F. reticulatum, F. equiseti, F oxysporum, F. semitectum, F. acuminatum, Rhizopus oryzae, A. niger, A. parasiticus, Cunningamella elegans, Syncephalestrum racemosum, Chaetomium indicum, Trichoderma sp. and Papulaspora immerse. Out of these 14 fungi, Trichoderma sp. and Fusarium solani showed highest % incidence while minimum % incidence recorded in case of Chaetomium indicum and Cunningamella elegans. The drug stored under the influence of different relative humidities viz. 30, 50, 75, 96 and 100% showed variation in % of occurrence as well as deterioration of the chemical constituents such as proteins, phenols, alkaloids and glycosides. The drug stored under 96 and 100% RH showed maximum deterioration of chemical constituents.

Key Words: Deterioration, Chemical constituents, Mycoflora, *Oroxylum indicum*

INTRODUCTION

Oroxylum indicum Vent. is commonly called as "Shivnak", "Shyonak", "Sonpatha", "Arlu", "Sauma", "Snapatha", "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-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 [1]. The root bark is used in fever, bronchitis, intestinal worms, leucoderma asthma, inflammation and troubles [2]. In many cases, chemical substances in plant medicine serve as the molecules of plant defense against microorganisms. However, several of these constituents possess medicinal properties [3]. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterol etc. Proteins are the critical factors in functioning of living cells. Phenol is a hydrocarbon compound that they have an important role in development of plant drugs. Alkaloids are a chemically heterogeneous group of natural substances and they show an extraordinary spectrum of pharmacological activities. Glycosides show therapeutically effects on humans and animals. The 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.The main chemical constituents in herbal drugs are alkaloids, sugars, phenols, proteins, flavonoids and glycosides. So it is important to study the influence of different relative humidity and different incubation days for detection of changes in selected chemical constituents in the roots of drug plant *O. indicum.*

MATERIAL AND METHODS

The root samples of drug *O. indicum* were collected from different localities, then they were brought to the laboratory in polyethylene bags to avoid aerial other contaminations. Agar plate method and Blotter test as recommended by International Seed Testing Association [4] were done for isolation of mycoflora associated with roots. 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, 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 references, such as Raper and Thom [5], Barnet and Hunter [6]; Thom and Raper [7]; Booth [8] and Nelson *et al.* [9]. Some parts of washed root samples were dried in oven and

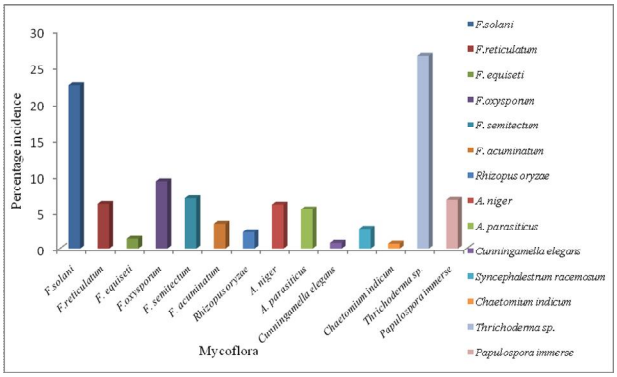
powdered by grinder and were used for biochemical analysis. Quantitative estimation of chemical constituents was carried out from first day to 90th days of incubation by standard procedure described by Lowry *et al*. [10] for total protein**,** Singh *et al*. [11] for total phenols, Harborne [12] for total alkaloids and Kokate *et al*. [13] for glycosides. 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 AND DISCUSSION

Total 14 fungi were isolated from roots of *Oroxylum indicum*; *Fusarium solani*, *F. reticulatum*, *F. equiseti*, *F oxysporum*, *F. semitectum*, *F. acuminatum*, *Rhizopus oryzae*, *A. niger*, *A. parasiticus*, *Cunningamella elegans*, *Syncephalestrum racemosum*, *Chaetomium indicum*, *Trichoderma* sp. and *Papulaspora immerse.*

The percentage incidence of all above 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%, *Cunningamella elegans* 0.8%, *Chaetomium indicum* 0.69%, *Trichoderma* sp. 26.52% and *Papulaspora immerse* 6.73%. In all isolated fungi; *Trichoderma* sp. found in high percentage incidence followed by *Fusarium solani*, while, *Chaetomium indicum* and *Cunningamella elegans* observed in very less percentage incidence (Graph 1).

Graph-1: Percentage incidence of mycoflora associated with the root o f *Oroxylum indicum*



The roots of *O. indicum* contained 42.91% at the first days. They stored at different RH 30, 50, 75, 96 and 100% RH, a gradual deterioration observed from first day to 90 days of incubation under all tested relative humidity. At 30, 50, 75, 96 and 100% RH after 90 days of storage period showed 37.22, 35.13, 33.33, 31.25 and 30.27% (Table 1).

Table 1: Deterioration of proteins content (mg/100mg) in root of *Oroxylum indicum* at different relative humidities

Incubation days	Control	30%	50%	75%	96%	100%
1 day	42.91±0.13	42.91±0.13	42.91±0.13	42.91±0.13	42.91±0.13	42.91±0.13
15days	42.91±0.16^{d}	42.77±0.21^{d}	42.63±0.21^{cd}	42.22±0.41^{bc}	41.66±0.13^{b}	40.41±0.48^{a}
30days	42.91±0.21^{c}	42.08±0.36^{c}	41.80±0.13^{c}	40.13±1.32^{b}	38.75±0.08^{a}	37.91±0.28^{a}
45 days	42.91±0.13^{e}	40.13±1.32^{d}	38.75±0.08^{c}	37.5±0.21^{bc}	36.11±0.60^{b}	34.44±0.65^{a}
60 days	42.91±0.48^{e}	40.27±0.55^{e}	38.88±0.36^{c}	36.25±0.72^{b}	34.72±0.28^{ab}	33.33±0.24^{a}
75 days	42.91±0.13^{d}	39.16±0.68^{d}	37.22±0.78^{d}	34.72±0.84^{b}	32.22±0.73^{a}	31.52±0.48^{a}
90 days	42.91±0.21^{e}	37.22±0.50^{d}	35.13±0.65^{c}	33.33±0.34^{b}	31.25±0.48^{a}	30.27±0.13^{a}

Data analysis of variance indicates that deterioration of total protein contents is under influence of incubation days and relative humidity and it is significant at 5% level significance (P-value <0.05).

The fresh sample of *O. indicum* stored at various relative humidity and different incubation days for 90 days. Minimum reduction in total phenols amount showed in case of 30% RH, after 15 days of incubation period total amount of phenols observed 5.51%, samples under 100% RH increasing in total phenols amount to 5.41% as compared to 96% RH observed but after that gradually decreased to 4.76 % after 90 days of incubation. Maximum deterioration observed under 96 and 100 % RH after 60 days of incubation days. These values reduced to 4.80, 4.66 % after 60 days of incubation period; total phenols value more deteriorated to 4.35 and 4.27% % after 90 days of incubation under 96 and 100 % RH, respectively (Table 2).

Table 2: Deterioration of total phenols content (mg/100mg) in root of *Oroxylum indicum* at different relative humidities

Incubation days	Control	30%	50%	75%	96%	100%
1 day	5.51±0.030	5.51±0.030	5.51±0.030	5.51±0.030	5.51±0.030	5.51±0.030
15days	5.51±0.019^{c}	5.51±0.011^{b}	5.51±0.09^{ab}	5.47±0.063^{ab}	5.37±0.079^{ab}	5.41±0.040^{a}
30days	5.51±0.039^{b}	5.49±0.074^{a}	5.33±0.019^{a}	5.27±0.30^{a}	5.13±0.059^{a}	5.05±0.11^{a}
45 days	5.51±0.019^{d}	5.33±0.079^{c}	5.27±0.070^{c}	5.05±0.069^{b}	4.92±0.022^{ab}	4.86±0.10^{a}
60 days	5.51±0.019^{e}	5.17±0.10^{d}	5.07±0.03^{d}	5±0.040^{c}	4.80±0.04^{b}	4.66±0.049^{a}
75 days	5.51±0.03^{d}	5.11±0.11^{d}	4.94±0.030^{c}	4.86±0.1^{c}	4.54±0.04^{b}	4.33±0.10^{a}
90 days	5.51±0.030^{e}	4.76±0.03^{d}	4.88±0.070^{d}	4.56±0.10^{c}	4.35±0.085^{b}	4.27±0.030^{a}

Data analysis of variance indicates that deterioration of total phenol contents is under influence of incubation days and relative humidity and it is significant at 5% level significance (P-value <0.05).

The sample of *O. indicum* stored at various relative humidity and different incubation days for 90 days. Minimum reduction in total alkaloid amounts showed in case of 30 % RH, after 15 days of incubation period total amount of alkaloids observed 34.89 % in samples which deteriorated to 34.58 after 90 days of incubation. Maximum deterioration observed under 96 and 100% RH after 60 days of incubation days. These values reduced to 34.47 and 34.43%, after 60 days of incubation period; total alkaloids value more deteriorated to 34.23, 34.15% after 90 days of incubation under 96 and 100 % RH, respectively (Table 3).

Table 3: Deterioration of total alkaloids content (mg/100mg) in root of *Oroxylum indicum* at different relative humidities

Incubation days	Control	30%	50%	75%	96%	100%
1 day	34.89±0.36	34.89±0.36	34.89±0.36	34.89±0.36	34.89±0.36	34.89±0.36
15days	34.89±0.49^{c}	34.89±0.14^{b}	34.89±0.90^{b}	34.87±0.42^{a}	34.85±0.41^{a}	34.83±0.40^{a}
30days	34.89±0.46^{c}	34.99±0.14^{b}	34.83±0.17^{bc}	34.80±0.39^{b}	34.72±0.11^{a}	34.63±1.12^{a}
45 days	34.87±0.42^{c}	34.82±0.46^{c}	34.77±0.39^{c}	34.68±0.36^{a}	34.61±0.42^{a}	34.54±0.42^{a}
60 days	34.88±0.047^{c}	34.74±0.18^{c}	34.65±0.39^{c}	34.54±0.17^{ab}	34.47±0.45^{a}	34.43±0.71^{a}
75 days	34.87±0.35^{c}	34.67±0.47^{c}	34.57±0.35^{b}	34.45±0.41^{a}	34.37±0.11^{a}	34.29±0.46^{a}
90 days	34.85±0.43^{c}	34.58±0.39^{b}	34.50±0.48^{b}	34.30±0.39^{ab}	34.23±1.04^{a}	34.15±0.14^{a}

Data analysis of variance indicates that deterioration of total alkaloid contents is under influence of incubation days and relative humidity and it is significant at 5% level significance (P value <0.05).

Fresh roots of *O. indicum* stored under different relative humidity and incubation days for observation of change in total glycosides content. The control samples in fresh and market sample contained 9.12%, this value deteriorated to 8.95% after 90 days of incubation. In case of 75% RH also observed the deterioration of glycosides 9.12, 9.11, 9.093, 9.02, 8.93, 8.89% after 15, 30, 45, 60, 75 and 90 days of incubation. Maximum reduction in total glycosides value observed in cases of 96 and 100% RH, after 30, 60 and 90 days of incubation in both of samples, total glycosides amount observed 9.10, 9.016, 8.82 (96 % RH); 9.096, 8.97, 8.79% (100 % RH) (Table 4).

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

Incubation days	Control	30%	50%	75%	96%	100%
1 day	9.12±1.12	9.12±1.12	9.12±1.12	9.12±1.12	9.12±1.12	9.12±1.12
15days	9.12±1.53^{c}	9.12±1.54^{c}	9.12±1.54^{b}	9.12±1.55^{a}	9.12±1.55^{a}	9.12±1.54^{a}
30days	9.12±1.53^{c}	9.12±1.54^{c}	9.12±1.53^{b}	9.11±1.54^{a}	9.10±1.55^{a}	9.096±0.015^{a}
45 days	9.12±1.52^{c}	9.10±0.044^{b}	9.10±0.043^{b}	9.093±0.52^{a}	9.06±1.29^{a}	9.03±1.45^{a}
60 days	9.12±1.52^{c}	9.066±0.073^{b}	9.03±0.075^{ab}	9.02±0.60^{a}	9.016±1.11^{a}	8.97±1.17^{a}
75 days	9.12±1.51^{c}	9.01±1.5^{b}	8.98±0.11^{a}	8.93±0.11^{a}	8.88±1.076^{a}	8.85±0.20^{a}
90 days	9.12±1.52^{c}	8.95±1.47^{b}	8.93±1.52^{ab}	8.89±0.016^{a}	8.82±1.035^{a}	8.79±0.12^{a}

Data analysis of variance indicates that deterioration of total glycoside contents is under influence of incubation days and relative humidity and it is significant at 5% level significance (P value <0.05).

Experimental result revealed that there was gradual depletion in alkaloids, glycosides, protein and phenols concentration under different relative humidity. Biodeterioration of selected chemical constituents might be due to enzymatic degradation into simpler components which are subsequently utilized by mycoflora for their growth [14,15]. These increase in total phenols after 15 days and under 100% RH, might be due to the enzymatic release, enhanced synthesis through shikimic acid pathway or production by parasite. Degradation of valuable secondary metabolites of stored plant drugs due to fungal infestation have been reported by some earlier workers[16-21] and these reports support our work in deterioration of total phenols.

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