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RESEARCH ARTICLE

EFFECT OF FUNGI ASSOCIATED WITH ROOTS OF *Aegle marmelos* LINN. (BAEL) ON CHANGES OF CARBOHYDRATES CONTENTS

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ABSTRACT

The plant *Aegle marmelos* Linn. belongs to family "Rutaceae" known as Bael is the accepted source of the drug and it is found throughout of India. The root of *Aegle marmelos* was selected for the present investigation**.** The roots stored at different relative humidities 30, 50, 75, 96 and 100% RH for 90 days. In the present study, total 20 fungal species were isolated from root samples. *Fusarium solani* (22.25%) showed highest percentage incidence. Quantitative estimation of carbohydrate relation to association of fungi was done. Maximum deterioration of carbohydrate contents and percentage incidence of fungi in the roots was showed at above 96% RH.

INTRODUCTION

Aegle marmelos commonly known as “Bael”, is an digenous tree. It is an Indian native plant, widely dispersed naturalised and used through Asia, it grows to height of 12 m. All the plant part used but generally not together. The root is an ingredient in the Dashmoola and used in Ayurvedic and other traditional medicinal system. The root is sweet and the decoction of the root, root-bark and sometimes the stem-bark, cures fevers due to "tridosha" pain in the abdomen palpitations of the heart, urinary troubles, hypochondriasis, melancholia removes "vata" "pitta" and "kopha". The roots also are a stringent, cooling, carminative, laxative, restorative, febrifuge and stomachic and they used in colitis, dysentery, diarrhoea, flatulence difficult micturition, fever, vomiting and colic. A large number (more than 110) of individual chemical constituents have been identified in various part of *A. marmelos*. Shoeb *et al*. (1973) have been observed the root of *A*. *marmelos* contain lupeol, skimmianine auroptene, marmin and umbelliferone, coumarins alkaloids, psoralen, scopoletin, tembamide and skimming. acetone and methanol extracts of the leaves of *A*. *marmelos* have phenol, alkaloids and flavanoids and also, methanol extract of the leave has antibacterial activity (Ulahannan *et al*., 2008). 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.

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Therefore, in this present study aimed to study mycoflora associated with Bael roots under influence of different relative humidity (RH) under storage, and also observed the changes in total sugar (TS), reducing sugar (RS) and non reducing sugar (NRS) of Bael roots by 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±3˚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 60th 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. Non-reducing sugar was estimated by subtracting the value of reducing sugar from total sugar.

Table1: Percentage incidence of fungal isolated from roots of *Aegle marmelos* stored at various relative humidity

Mycoflora	RH	30%				50%				75%				96%				100%			
	Days	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60
F. solani	-	0.34	0.41	0.61	0.75	0.55	0.75	1.03	1.23	0.68	1.03	1.16	1.37	0.82	1.23	1.44	1.92	1.03	1.51	1.92	2.47
F.oxysporum	-	-	-	-	-	-	-	0.27	0.41	0.13	0.34	0.48	0.61	0.27	0.55	0.75	1.031	0.41	0.75	1.031	1.23
F.lateritum	-	-	-	-	-	-	-	-	0.34	-	0.27	0.48	0.61	0.48	0.61	0.75	1.031	0.48	0.82	1.10	1.23
F. acuminatum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.20	0.34	0.48	-	0.27	0.55	0.82
F. sambucinum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.13	-	0.06	0.20	0.27
A. flavus	-	-	-	-	-	-	-	-	-	0.06	0.20	0.27	0.34	0.13	0.41	0.48	0.61	0.20	0.48	0.75	1.03
A. niger	-	-	-	-	-	-	-	-	0.27	-	0.13	0.34	0.48	0.20	0.41	0.61	0.75	0.34	0.48	0.82	1.031
Monilia sitophila	-	-	-	-	-	-	-	0.13	0.20	-	-	0.27	0.41	-	-	-	0.48	-	0.34	0.48	0.75
Sordaria fimicola	-	-	-	-	-	-	-	-	0.20	-	-	-	0.20	-	-	0.20	0.34	-	-	0.41	0.61
Theilavia terricola	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.06	0.20	0.06	0.06	0.27	0.48
Rhizopus oryzae	-	-	-	-	-	-	-	-	0.27	-	-	-	0.34	-	-	0.06	0.34	0.13	0.41	0.48	0.61
Didymostilbe sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.06	0.13	0.20	0.06	0.20	0.27	0.41
Acremonium sp.	-	-	-	-	-	-	-	-	0.13	-	-	0.20	0.27	-	-	0.27	0.41	0.06	0.20	0.34	0.55
Thrichoderma sp.	-	-	-	-	-	-	-	-	-	-	-	-	0.13	-	-	0.13	0.27	-	0.20	0.48	0.75
Curvularia lunata.	-	-	-	-	-	-	-	0.20	0.34	-	0.20	0.48	0.61	0.27	0.41	0.55	0.031	0.41	0.55	0.75	0.96
Nigrospora oryzae	-	-	-	-	-	-	-	0.34	0.48	0.20	0.34	0.55	0.61	0.34	0.55	0.75	0.96	0.41	0.61	0.82	1.23
Chaetomium globosum	-	-	-	-	-	-	-	0.06	0.13	-	-	0.13	0.20	-	0.20	0.34	0.61	0.27	0.34	0.55	0.75
Achlya sp.	-	-	-	-	-	-	-	-	-	-	-	0.06	0.13	-	0.20	0.27	0.34	0.06	0.20	0.48	0.68
Papulospora immerse	-	-	-	-	-	0.06	0.20	0.34	0.48	0.13	0.27	0.55	0.75	0.20	0.48	0.61	1.031	0.27	0.55	0.82	1.23
Aphanomyces sp.	-	-	-	-	-	-	-	-	0.06	-	-	0.06	0.13	-	0.06	0.20	0.34	0.13	0.41	0.61	0.89
Total % incidence	-	0.34	0.61	0.61	0.75	0.61	0.95	2.37	4.54	1.2	2.78	5.03	7.19	2.71	5.37	7.94	11.50	4.32	8.44	13.13	17.98

Table 2: Deterioration of total carbohydrate content (mg/100mg) in root of *Aegle marmelos* stored at different relative humidities

Incubation days	control	30%	50%	75%	96%	100%
1 day	65.31±0.19	65.31±0.19	65.31±0.19	65.31±0.19	65.31±0.19	65.31±0.19
15days	65.31±0.072 ^{d}	65.15±0.12 ^{c}	64.52±0.12 ^{c}	63.55±0.62 ^{b,c}	62.66±0.59 ^{a}	62.20±0.47 ^{a}
30days	65.31±0.31 ^{d}	62.37±0.12 ^{c}	60.73±0.12 ^{c}	59.59v0.33 ^{b,c}	58.20±0.12 ^{a}	56.81±0.12 ^{a}
45 days	65.31±0.26 ^{d}	58.08±0.95 ^{c}	56.48±0.16 ^{c}	54.29±0.25 ^{c}	52.90±0.25 ^{a,b}	51.97±0.38 ^{a}
60 days	65.31±0.26 ^{d}	55.17±0.21 ^{c}	54.41±0.12 ^{c}	53.99±0.14 ^{b}	51.55±0.38 ^{a}	50.50±0.37 ^{a}
75 days	65.31±0.19 ^{d}	53.32±0.31 ^{c}	52.81±0.19 ^{c}	51.93±0.44 ^{c}	50.63±0.25 ^{a}	49.49±0.70 ^{a}
90 days	65.31±0.26 ^{d}	50.42±0.19 ^{c,d}	49.91±0.38 ^{c}	48.77±0.52 ^{b,c}	47.89±0.29 ^{a}	47.05±0.26 ^{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 reducing sugar content (mg/100mg) in root of *Aegle marmelos* stored at different relative humidities

Incubation days	control	30%	50%	75%	96%	100%
1 day	5.42±0.045	5.42±0.045	5.42±0.045	5.42±0.045	5.42±0.045	5.42±0.045
15days	5.42±0.23 ^{d}	5.42±0.052 ^{c,d}	5.29±0.026 ^{c}	5.15±0.045 ^{b,c}	4.97±0.11 ^{a,b}	4.93±0.20 ^{a}
30days	5.42±0.16 ^{d}	5.24±0.045 ^{c,d}	5.02±0.069 ^{c}	4.81±0.09 ^{b,c}	4.66±0.026 ^{a}	4.57±0.026 ^{a}
45 days	5.42±0.078 ^{d}	5.15±0.13 ^{c,d}	5.02±0.31 ^{b,c}	4.86±0.032 ^{b}	4.63±0.095 ^{a}	4.43±0.045 ^{a}
60 days	5.42±0.045 ^{d}	5.06±0.069 ^{c,d}	4.93±0.090 ^{c}	4.70±0.045 ^{b}	4.38±0.17 ^{a}	4.02±0.069 ^{a}
75 days	5.42±0.045 ^{d}	4.66±0.090 ^{c,d}	4.43±0.069 ^{c}	4.07±0.094 ^{b}	3.61±0.045 ^{a}	3.57±0.11 ^{a}
90 days	5.42±0.026 ^{d}	4.20±0.11 ^{d}	3.89±0.20 ^{b,c}	3.66±0.15 ^{b}	3.39±0.069 ^{a,b}	3.21±0.045 ^{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

Table4: Deterioration of non reducing sugar content (mg/100mg) in root of *Aegle marmelos* stored at different relative humidities

Incubation days	control	30%	50%	75%	96%	100%
1 day	59.89±0.2	59.89±0.22	59.89±0.22	59.89±0.2	59.89±0.2	59.89±0.2
15days	59.89±0.2 [d]	59.75±0.17 [d,c]	59.57±0.62 [c]	58.14±0.5 [b]	57.64±0.5 [a]	57.39±0.4 [a]
30days	59.89±0.2 [d]	57.12±0.42 [d,c]	55.60±0.14 [c]	54.75±0.4 [b]	53.53±0.1 [a]	52.26±0.1 [a]
45 days	59.89±0.3 [d]	52.91±0.92 [d]	51.45±0.33 [c]	49.40±0.2 [b]	48.30±0.3 [a]	47.54±0.4 [a]
60 days	58.89±0.2 [d]	50.09±0.21 [d]	49.55±0.35 [c]	49.29±0.1 [b]	47.29±0.2 [a]	46.49±0.4 [a]
75 days	58.86±0.2 [d]	48.66±0.22 [d,c]	48.37±0.23 [c]	47.71±0.7 [b]	47.01±0.27 [a]	45.87±0.60 [a]
90 days	58.80±0.25 [d]	46.23±0.095 [d,c]	46.073±0.25 [c,d]	45.1±0.52 [c,b]	44.5±0.22 [a,b]	43.84±0.27 [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

Statistical 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

In the present study 20 fungi were isolated from the roots of *Aegle marmelos*. The fungi include: *Fusarium solani*, *F. oxysporum*, *F. lateritum, F. acuminatum, F. sambucinum*, *A. niger, A. flavus*, *Monilia sitophila*, *Sordaria fimicola*, *Theilavia terricola*, *Rhizopus oryzae*, *Didymostilbe* sp., *Acremonium* sp., *Thrichoderma* sp., *Curvularia lunata*, *Nigrospora oryzae*, *Chaetomium globosum*, *Achlya* sp., *Papulospora immerse* and *Aphanomyces* sp. The samples stored at 75, 96 and 100% relative humidity after 45 days of incubations showed higher percent incidence of fungi (Table 1). On 60th days of incubation total percentage of isolated fungi were recorded 0.7, 4.54, 7.19, 11.5 and 17.9 % stored at 30, 50, 75, 96 and 100% RH respectively. Incubation days and relative humidity in association of isolated fungi, influenced on carbohydrates amounts. The roots stored under higher relative humidity (above 75% RH) and stored under maximum incubation days (above 60^{th} days) showed maximum deterioration of chemical constituents. The roots of Bael contained 65.31% total sugar, which, gradually decreased to 47.05% at 90 days of storage at 100% RH. The maximum deterioration observed at the end of 90^{th} days. In case 30, 50, 75 and 96% RH observed percentage incidences of total sugars observed 50.42, 49.91, 48.77 and 47.89% of deterioration respectively (Table 2).

The root of *Aegle marmelos* contained 5.42% reducing sugar which gradually reduced under different incubation days when, on 90^{th} days of incubation, percent age incidence in roots stored at different relative humidities 30, 50, 75, 96 and 100%, were reduced to 4.20, 3.89, 3.66, 3.39 and 3.21 %, respectively (Table 3). The control samples contained 59.89% non reducing sugar, which was reduced to 43.84 % on 90^{th} days of incubation and under different relative humidities (Table4). Analysis variance showed the effect of relative humidity and incubation days in the reduction of total sugar, reducing sugar and non reducing sugar contents were significant at 5% of significance P (sig) <0.05.

DISCUSSION

Carbohydrates are a good source of carbons for fungi. Generally fungi associated with plants parts are converted carbohydrates into simpler forms by the enzymatic activities of the fungi and subsequently utilized by them. A remarkable loss in sugar contents of drug plant parts due to spoilage of fungi under storage reported by several researchers (Kabnoorkar and Deokule, 2009; Kumar and Nair, 1981; Singh and Bedi, 1976) which support our result in this investigation.

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