



## STUDIES ON EFFECT OF RELATIVE HUMIDITY ON FUNGAL GROWTH AND DETERIORATION OF TOTAL ALKALOIDS IN FRESH AND MARKET ROOTS OF DRUG *URARIA PICTA* DC.

M Rashidi\* S S Deokule and M Abyari

Department of Botany, University of Pune, Pune, India

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\*E-mail: rashidi129@gmail.com

### ABSTRACT

In the present study, 26 fungi have been isolated from the fresh and market roots of drug *Uraria picta* Dc. Fungi *F. solani* with 31.5% observed in highest percentage incidence from genuine samples but in the market samples fungi *A. niger* recorded with 14.07 % incidence to be dominant fungi. The root samples stored at different relative humidities 30, 50, 75, 96 and 100% RH in different incubation days. Effect of growth and association of fungi under selected RH and incubation days on quantitative estimation of total alkaloids was done. The result revealed high percentage incidence of fungi and rate of reduction of total alkaloids in samples were noted at above 75% RH. More deterioration of total alkaloids recorded in case of market samples as compare to genuine samples. Analysis of variance also showed that the effect of relative humidity and incubation days on biodeterioration of total alkaloid amount were significant.

**Key words:** *Uraria picta*, fungi, deterioration, total alkaloids, relative humidity

### INTRODUCTION

*Uraria picta* is a suffruticose sparingly branched perennial herb, it is one part of the important constituents of “Dashmula” a well established Ayurvedic drug for treating general fatigue, oral sores and several gynaecological disorders. The whole plant is medicinally important and is used by certain Adivasi and native tribes<sup>6</sup>. Traditionally, the plant is used as an antidote to the venom of a dangerous Indian snake, *echis carinata*<sup>7</sup>.

The fruits and pods are effective against oral sores in children and the roots are aphrodisiac in nature. They are mainly used for fracture healing properties; the decoction is useful in cough, chills and fevers<sup>7,10</sup>. Microbial contamination are responsible for reduction of quality of medicinal plants which they happens in favourable condition, there are a few reports focused on associated fungi with herbal drugs and their effects on changes of chemical constituents in herbal drugs due to spoilage of fungi. Therefore our attempt has been made to investigate fungi associated with fresh and stored drug roots of *U. picta* and effect of different range of RH and incubation period on incidence of fungi and changes of total alkaloids.

### MATERIAL AND METHODS

The fresh root of drug *Uraria picta* were collected in healthy, flowering and fruiting conditions from different places of Pune, India. Besides the genuine root drug, market survey has been carried out for the collection of the marketed roots from various Shopkeepers, storehouse and Kashtaushdhis of Mumbai and Pune. They were brought to the laboratory in polyethylene bag to avoid aerial contamination. The organ form of roots cut to small pieces and sterilized with 2% sodium hypochlorite solution and thoroughly washed with sterilized distilled water. Blotter test and agar plate method as recommended by International Seed Testing Association (1966) were adopted for isolation of mycoflora associated with roots. In order to evaluate of biochemical changes of alkaloids contents related to mycoflora associated, the root samples were stored in small muslin clothes bags under 30, 50, 75, 96 and 100 % RH for 90 days in the room temperature. At an interval of 15 days, root samples were taken out and thoroughly washed with distilled water and plated in Petri plates. The percentage incidence of mycoflora

was recorded from first day to 60<sup>th</sup> day of storage. Fungi were identified by using different keys<sup>1,8,9</sup>. Some parts of washed drug samples were dried in oven and powdered by grinder to carried out the estimation of total alkaloids in genuine and marketed samples, the changes in percentage of total alkaloids was estimated by standard procedure<sup>4</sup>. Data from the experiment were analyzed by SPSS software in which statistical significance was determined at 0.05 % probability levels.

### RESULTS

Total 16 fungi were isolated from fresh roots of *Uraria picta* (Table1). *F. solani* with 31.5% observed the highest percentage incidence following by *F. semitectum* 17.2%, *A. niger* 10.23%, *Thrichoderma* sp. 8.82% and *F. oxysporum* 6.26 %. Other isolated fungi showed different percentage incidence: *F. comptocera* 2.91%, *F. equiseti* 1.5%, *A. fumigatus* 2.2%, *Mucor praini* 3.44%, *Papulospora immerse* 5.03%, *Sclerotium* sp. 1.41%, *Thraustotheca* sp. 5.91%, *Theilavia terricola* 1.5% and *Rhizoctonia solani* 0.79%. The fungi *Neurospora sitophila* and *Cunningamella elegans* with 0.79 and 0.88 % incidence showed less percentage incidence. From the stored roots of this drug 14 fungi were isolated (Table2). *A. niger*, *A. fumigatus*, *A. ochraceus*, *A. parasiticus*, *A. oryzae*, *Ch. globosum*, *Ch. indicum*, *Thrichoderma* sp., *Syncephalestrum racemosum*, *Rhizopus oryzae*, *Scytallidium thermophilum*, *Nigrospora oryzae* and *Monilia sitophila*. The fungi *A. niger* found in 14.07%, while *Scytallidium thermophilum*, *A. parasiticus*, *Thrichoderma* sp., showed 13.51, 12.51 and 10.16 percentage incidence respectively. *A. ochraceus*, *A. oryzae*, *R. oryzae*, *A. fumigatus* and *Ch. globosum* occurred in 9.94%, 7.37%, 6.92%, 6.36% and 4.46%, respectively. *Monilia sitophila* and *Acremonium* sp. showed same percentage incidence 4.24%. Other fungi are observed in less percentage incidence which includes: *Syncephalestrum racemosum* and *Ch. indicum* showed same percentage incidence 2.01%.

The fresh roots of *Uraria picta* stored at different relative humidity and after 60 days of incubation, total percentage incidence of mycoflora under selected relative humidity recorded (Table3). After 15 days of incubation under 30, 50, 75, 96 and 100% RH, total percentage incidence of all isolated fungi observed 1.059, 1.58, 1.67, 3.35 and 4.50 % ,

after 60 days of storage they are increased to 3.70, 4.94, 7.33, 10.32 and 14.29 %.

Root samples of this drug which collected from market, also analysed for effect of relative humidity on fungal growth and total percentage incidence of mycoflora recorded (Table 4). In 30 % RH, after the storage of 45 and 60 days the total percentage incidence recorded 1.11 and 2.11 %, under 75 % RH, after 15 to 60 days total percentage incidence from 0.89% is increased to 7.039 %, under 96 % RH, the percentage incidence observed after 15, 30, 45 and 60 day 2.23, 4.46, 8.15 and 11.39 % respectively, under 100 % RH the percentage incidence observed 5.47, 9.83, 13.74 and 18.54 %.

Fresh sample of *U. picta* stored under different relative humidity and storage period shows the different range of deterioration of total alkaloids. The control sample of this drug contains 19.45 % total alkaloids, which after 30, 50, 75, 96 and 100% RH, after 15 days of incubation observed 19.45, 19.44, 19.44, 19.41, 19.38%, these values reduced to 19.16, 19.056, 18.91, 18.83, 18.71% after 90 days of incubation period (Table 5).

Market samples contain 19.31 % of total alkaloid in control samples. Minimum reduction in total alkaloids amount shows after 15 days of incubation days and under 30 and 50 % RH, 19.31 and 19.30% which these amount after 30, 60, 90 days of incubation deteriorated to 19.22, 19.14; 18.92, 18.82%; 18.50, 18.65 % respectively, in case of 96 and 100 % RH, after 15 days of storage total value of alkaloid amount observed 19.23, 19.14% which at the end of 90 days, reduced to 18.42 and 18.28% (Table 6).

The results were analyzed statistically which analysis of variance showed that relative humidities and incubation days on the deterioration of alkaloids contents were significant at 5% & 1% level of significance, P value (sig) < 0.01, 0.05.

## DISCUSSION

The traditional methods of collection, storage and marketing coupled with humid climatic condition make medicinal plant victim to the fungal infestation. Unscientific drying, storage methods and good environmental conditions favour association of various microbes with stored products. Fungi are very selective in their nutritional requirements. When they attack on host they influence the stored substances by absorbing them or by converting some of the complex forms into simpler ones. Reduction of total alkaloids amount under storage of different relative humidities due to spoilage of mycoflora may be due to their degradation into simpler forms by microbial enzymes which produce by fungi and their utilization as a source of energy for their growth. This was in accordance with the result of earlier workers<sup>2,3</sup>.

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**Table 1: Mycoflora associated with the fresh roots and their percentage incidence**

No	Isolated mycoflora	Percentage incidences
1	<i>F. solani</i>	31.5
2	<i>F. semitectum</i>	17.2
3	<i>F. oxysporum</i>	6.26
4	<i>F. comptocera</i>	2.91
5	<i>F. equiseti</i>	1.50
6	<i>A. niger</i>	10.23
7	<i>A. fumigatus</i>	2.20
8	<i>Mucor praini</i>	3.44
9	<i>Cunningamella elegans</i>	0.88
10	<i>Papulospora immerse</i>	5.03
11	<i>Sclerotium</i> sp.	1.41
12	<i>Thrichoderma</i> sp.	8.82
13	<i>Thraustotheca</i> sp.	5.91
14	<i>Theilavia terricola</i>	1.5
15	<i>Neurospora sitophila</i>	0.35
16	<i>Rhizoctonia solani</i>	0.79

**Table 2: Mycoflora associated with the market roots and their percentage incidence**

No	Isolated mycoflora	Percentage incidences
1	<i>Aspergillus niger</i>	14.07
2	<i>A. fumigates</i>	6.36
3	<i>A. ochraceus</i>	9.94
4	<i>A. parasiticus</i>	12.52
5	<i>A. oryzae</i>	7.37
6	<i>Nigrospora oryzae</i>	2.12
7	<i>Scytallidium termophilum</i>	13.51
8	<i>Monilia sitophila</i>	4.24
9	<i>Rhizopus oryzae</i>	6.92
10	<i>Ch. Globosum</i>	4.46
11	<i>Ch. Indicum</i>	2.011
12	<i>Acremonium</i> sp.	4.24
13	<i>Thrichoderma</i> sp.	10.16
14	<i>Syncephalestrum racemosus</i>	2.011

**Table 3:** Percentage incidence of fungal isolated from the fresh roots of *Uraria picta* stored at various relative humidity

Microflora	con	30%	45	60	15	30	45	60	15	30	45	60	15	30	45	60	100%
<i>F. solani</i>	0.44	0.88	1.05	1.23	1.41	0.88	0.97	1.14	1.5	0.97	1.5	1.67	1.85	1.32	1.67	2.64	1.58
<i>F. dematium</i>	0.08	0.17	0.44	0.79	0.97	0.26	0.44	0.61	1.05	0.61	0.79	0.88	1.32	0.88	0.97	1.23	1.58
<i>F. oxysporum</i>	-	-	-	-	-	0.08	-	0.08	0.17	0.26	-	0.35	0.7	0.17	0.35	0.44	0.88
<i>F. compacta</i>	-	-	-	-	-	-	-	-	-	-	-	0.17	0.26	-	0.08	0.17	0.35
<i>F. equisetii</i>	-	-	-	-	-	-	-	-	-	-	-	0.08	0.17	0.17	0.17	0.17	0.61
<i>A. niger</i>	-	-	-	-	-	0.17	0.26	0.8	0.26	0.35	0.08	0.17	0.44	0.61	0.35	0.7	0.97
<i>A. flaviglobus</i>	-	-	-	-	-	0.08	-	-	0.17	-	-	0.17	0.26	-	0.17	0.26	0.44
<i>Mucor pravini</i>	-	-	-	-	-	-	-	-	0.08	-	-	0.17	-	-	0.08	0.26	0.7
<i>Cunninghamella elegans</i>	-	-	-	-	-	-	-	-	-	-	-	0.08	-	-	0.17	-	0.44
<i>Populicystopora</i>	-	-	-	-	-	0.35	-	-	0.26	0.44	-	0.17	0.35	0.52	-	0.61	0.17
Diminuta	-	-	-	-	-	-	-	-	0.08	-	-	0.17	-	-	0.08	0.17	0.26
<i>Sclerotiorum</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.26
<i>Thrichoderma</i> sp.	-	-	-	-	-	0.26	0.35	-	0.17	0.44	-	0.35	0.52	0.61	0.26	0.35	0.79
<i>Trichocomaceae</i> sp.	-	-	-	-	-	0.26	-	-	0.16	0.44	-	-	0.26	0.44	0.08	0.17	0.35
<i>Tissularia</i> sp.	-	-	-	-	-	-	-	-	-	-	-	0.08	0.08	-	-	0.17	0.79
<i>Neurospora sitophila</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.08	-	0.17
<i>Rhizoctonia solani</i>	-	-	-	-	-	1.23	1.56	2.82	4.94	1.67	3.26	5.03	7.23	3.35	4.85	7.23	10.32
Total	0.52	1.05	1.5	2.47	3.7	1.23	1.56	2.82	4.94	1.67	3.26	5.03	7.23	3.35	4.85	7.50	10.94
																	14.29

**Table 4:** Percentage incidence of fungal isolated from the market roots of *Uraria picta* stored at various relative humidity

Microflora	con	30%	45	60	15	30	45	60	15	30	45	60	15	30	45	60	100%
<i>Aspergillus</i>	-	-	0.44	0.55	-	0.33	0.55	0.77	0.11	0.33	0.66	0.99	0.33	0.55	0.88	1.22	1.67
<i>niger</i>	-	-	-	-	0.11	-	0.11	0.11	0.33	-	0.22	0.44	0.55	0.11	0.33	0.44	0.66
<i>A. flavigens</i>	-	-	-	-	0.11	0.11	0.22	0.33	0.44	0.11	0.44	0.66	0.88	0.33	0.44	0.66	0.88
<i>A. ochraceus</i>	-	-	-	-	0.11	0.22	-	-	0.22	0.33	0.22	0.33	0.55	0.77	0.33	0.44	0.66
<i>A. parasiticus</i>	-	-	-	-	0.11	0.22	-	-	0.22	0.33	0.11	0.11	0.33	0.44	0.11	0.33	0.55
<i>A. oryzae</i>	-	-	-	-	0.11	0.11	-	-	0.22	0.33	0.11	0.11	0.33	0.44	0.11	0.44	0.55
<i>Nigrospora</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.22
<i>ononis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.66
<i>Scutellatium</i>	-	-	0.11	0.33	0.11	0.33	0.55	0.66	0.22	0.44	0.66	0.99	0.33	0.66	1.22	1.45	2.011
<i>Termitophilum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.99
<i>Montia</i>	-	-	-	-	0.11	-	-	0.11	-	0.11	0.22	0.33	-	0.22	0.44	0.66	0.11
<i>zizophila</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	0.11	0.22	0.33	-
<i>Rhizopus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	0.11	0.22	0.33	-
<i>ononis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.77
<i>On. globosum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.22
<i>On. indicum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.66
<i>Artemesia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.55
<i>sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.77
<i>Thrixocetoma</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.45
<i>sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.22
<i>Sporella</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.33
<i>Lamprolechia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.55
<i>Lampronia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.55
Total	-	-	-	-	1.11	2.11	0.22	1	2.34	3.91	0.89	2.34	4.8	7.03	2.23	4.46	8.15
																	13.74
																	18.54

**Table 5: Deterioration of total alkaloids content (mg/100mg) in root of *Uraria picta* (Fresh sample) at different relative humidities**

Incubation days	Control	30%	50%	75%	96%	100%
1 day	19.45±0.72	19.45±0.72	19.45±0.72	19.45±0.72	19.45±0.72	19.45±0.72
15days	19.45±0.73d	19.45±0.72c	19.44±0.69c	19.44±0.70ab	19.41±0.87a	19.38±0.72a
30days	19.44±0.71d	19.44±0.10c	19.39±0.72c	19.37±0.37ab	19.34±0.72a	19.26±0.78a
45 days	19.44±0.71d	19.38±0.72c	19.32±0.75c	19.28±0.80ab	19.16±0.78a	19.20±0.49a
60 days	19.43±0.74d	19.25±0.77c	19.19±0.77c	19.13±0.47ab	19.10±0.79a	19.063±0.79a
75 days	19.43±0.72d	19.22±0.47c	19.11±0.76cb	19.01±0.75b	18.94±0.80a	18.9±0.41a
90 days	19.43±0.71d	19.16±0.75c	19.056±0.78cb	18.91±0.52b	18.83±0.72a	18.71±0.78a

**Table 6: Deterioration of total alkaloids content (mg/100mg) in root of *Uraria picta* (Market sample) at different relative humidities**

Incubation days	Control	30%	50%	75%	96%	100%
1 day	19.31±0.77	19.31±0.77	19.31±0.77	19.31±0.77	19.31±0.77	19.31±0.77
15days	19.31±0.76d	19.31±0.78c	19.30±0.77c	19.26±0.80bc	19.23±0.81ab	19.14±0.72a
30days	19.31±0.75d	19.22±0.74c	19.14±0.84c	19.09±0.85bc	19±0.89ab	18.92±0.90a
45 days	19.30±0.74d	19.05±0.85c	18.96±0.79c	18.89±0.87b	18.82±0.81a	18.75±0.83a
60 days	19.30±0.74d	18.92±0.81c	18.82±0.88cb	18.71±0.87b	18.64±0.88a	18.56±0.91a
75 days	19.29±0.76d	18.80±0.84c	18.74±0.79cb	18.67±0.75b	18.59±0.80a	18.53±0.81a
90 days	19.28±0.76d	18.50±0.80c	18.65±0.78cb	18.56±1.52ab	18.42±2.72a	18.28±1.09a

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=0.01, 0.05

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