



## FUNGAL ASSOCIATION AND DETERIORATION OF CHEMICAL CONSTITUENTS OF *CULLEN* *CORYLIFOLIA* (LINN.) MEDIK. SEEDS

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In the present study, total 14 fungi were found associated with stored samples of *C. corylifolia* seeds such as, *Aspergillus niger*, *A. parasiticus*, *A. elegans*, *Rhizopus stolonifer*, *A. flavus*, *Chaetomium globosum*, *A. oryzae*, *Fusarium* sp., *Ch. spirale*, *Curvularia* sp., *A. terreus*, *Alternaria* sp., *Penicillium rubrum* and *Trichurus spiralis*. Out of these 14 fungi, *Aspergillus niger* and *A. parasiticus* showed highest % incidence while minimum % incidence recorded in case of *Penicillium rubrum*, *Alternaria* sp., & *Trichurus spiralis*. The drug stored under the influence of different relative humidities viz. 33, 55, 75, 96 and 100% showed variation in % of occurrence as well as biodeterioration of the chemical constituents such as sugars, proteins, phenols and glycosides. The drug stored under 96 and 100% RH showed maximum deterioration of chemical constituents.

**Key words:** chemical constituents, deterioration, fungi, % incidence.

The plant *Cullen corylifolia* (Linn.) Medik (= *Psoralea corylifolia* L.) commonly known as 'Bavchi' belongs to family 'Fabaceae' (Kirtikar and Basu 1999). The drug has been considered to be so efficacious in leprosy that it was given the name of 'Kushtanashini' means 'leprosy destroyer' (Nadkarni 1927). Seeds are used as antipyretic, anthelmintic, alexeteric. It is good for heart troubles, asthma, leucoderma, urinary discharges, heals, ulcers. It possesses purgative, stimulant and aphrodisiac properties. It improves appetite and good for scabies and biliousness. It cures the blood diseases (Kirtikar and Basu 1999). The seeds have some repute in native medicine as a remedy for certain skin diseases. *Psoralea* is recommended in the treatment of leprosy, psoriasis and inflammatory diseases of the skin (Kokate *et al.* 2002).

During harvesting, collection and storage these drugs may be contaminated with fungi. A few reports are available which support our study (Dutta and Roy 1987, Roy *et al.* 1987, Inman 1962, Marx *et al.* 1966, Wagner 1977, Rose, 1981, Maheshwari, 1987, Al-Juraifani, 2011, Sareen *et al.*, 2010, and Anyanwu, 2010). But the available information is very meager. Therefore, it has been decided to study the

fungus association and deterioration of chemical constituents of *Cullen corylifolia* seeds under the influence of different relative humidity.

### MATERIAL AND METHODS

The seeds of '*Psoralea*' were collected from different storehouse/ kashataushdis of Pune. It was brought to the laboratory in separate polyethylene bags to avoid aerial contamination. Blotter test method, as recommended by International Seed Testing Association (1966) was adopted for isolation of fungi. Agar plate method and surface washing methods were also used. The seeds were sterilized with 2% NaOCl solution before plating. In order to evaluate the chemical changes in relation to fungi associated, the samples were stored in small muslin cloth bags under different RH levels i.e. 33, 55, 75, 96, 100 % and at 28 °C temperature for 90 days (Wink and Sears 1950). At an interval of 15 days, samples were taken out and washed thoroughly in 100 ml sterilized glass distilled water. After that calculate the % incidence of fungi and then washed samples were dried in oven for chemical analysis. The changes take place in percentage of sugars, proteins, phenols and glycosides in the samples were estimated by

the methods of Nelson (1941), Lowry *et al.* (1951), Singh *et al.* (1978) and Kokate *et al.* (2002).

The % incidence of fungi was calculated by using the following formula –

$$\frac{\text{No. of colonies of a particular species}}{\text{Total no. of colonies of all the species}} \times 100$$

Total no. of colonies of all the species

## RESULTS AND DISCUSSION

14 fungal species were found to be associated with '*Psoralea*' seeds in varying percentages such as *Aspergillus niger* (19.2%), *A. parasiticus* (14.0%), showed higher % incidence followed by *A. elegans* (8.7%), *Rhizopus stolonifer* (8.7%), *A. flavus* (7.0%), *Chaetomium globosum* (7.0%), *A. oryzae* (5.2%), *Fusarium* sp. (5.2%), *Ch. spirale* (5.2%), *Curvularia* sp. (5.2%), *A. terreus* (5.2%), *Alternaria* sp. (3.5%), *Penicillium rubrum* (3.5%) and *Trichurus spiralis* (1.7%).

Seeds of *C. corylifolia* were stored under different relative humidity and observed the deterioration of sugar in each relative humidity (Table 1). The drug stored under 75 % RH after 15 days of incubation TS (Total sugar), RS (Reducing sugar) and NRS (Non reducing sugar) observed were 11.5, 5.1 and 6.4 % reduction and it shows more deterioration after 90 days of incubation period i.e. 9.8, 4.3 and 5.5 %. The drug stored under 96, 100 % RH, after 90 days of storage recorded maximum reduction in sugar contents.

Seeds of *C. corylifolia* contain 5.88 % total protein, this value decreases to 3.88 % results was depicted in Table 2. The drug stored at 75 % RH showed the gradual decrease in the protein value 5.04, 4.64 and 4.24 % while in 96 % RH showed 4.8, 4.32 and 3.96 % declination of protein after 30, 60 & 90 days of storage. In 100 % RH observed the maximum deterioration of protein i.e. after 30 days (4.76 %), 60 days (4.2 %) and 90 days (3.88 %),

respectively.

The total phenol in *C. corylifolia* was estimated to be 2.9 %. This value of phenol was deteriorated to 1.65 % under the influence of different relative humidity (Table - 3).

*C. corylifolia* seeds contain 2.75 % of glycoside (Table - 4). The drug stored at 75 % relative humidity observed 2.69 % of deterioration after the storage of 15 days. At 30, 60 and 90 days observed the 2.65, 2.56 and 2.50 % loss in glycoside while the drug stored at 96 %

RH recorded the gradual decrease in values of glycoside i.e. 2.67, 2.63, 2.57, 2.54, 2.51 and 2.48 %, after the storage of 15, 30, 45, 60, 75 and 90 days. Lastly the drug stored at 100 % RH also observed reduced value of glycoside. After the incubation of 30, 60, 90 days of storage it showed 2.61, 2.52, 2.45 % of deterioration of glycoside, respectively.

Analysis of variance shows that the effects of relative humidity and incubation periods in the reduction of sugars, proteins, phenols, and glycosides contents were significant at 1 % level of significance.

## CONCLUSIONS

The fungi isolated from seeds of '*C. corylifolia*' in various % incidence spoils the seeds severely and ultimately affects the deterioration of chemical constituents. During the storage (1 to 90 days and 33 to 100% RH) from the biochemical-analysis of '*Psoralea*' seeds it was observed that 75, 96 and 100% RH showed the significant reduction in the sugars, proteins, phenols and glycosides contents. The seeds stored at 33 and 55% RH showed minimum deterioration of chemical constituents. The maximum storage period is also responsible for the maximum association of fungi.

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Table 1. Deterioration of sugar ( mg/100mg ) in seeds of *C. corylifolia* under storage at different relative humidity.

Incubation Days	Control			33%			55%			75%			96%			100%		
	TS	RS	NRS	TS	RS	NRS	TS	RS	NRS	TS	RS	NRS	TS	RS	NRS	TS	RS	NRS
1	11.8	5.3	6.5	11.8	5.3	6.5	11.8	5.3	6.5	11.8	5.3	6.5	11.8	5.3	6.5	11.8	5.3	6.5
% Change over control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	11.8	5.3	6.5	11.8	5.3	6.5	11.8	5.3	6.5	11.5	5.1	6.4	11.3	5	6.3	11.1	4.9	6.2
% Change over control	0	0	0	0	0	0	0	0	0	2.5	3.7	1.5	4.2	5.6	3.0	5.9	7.5	4.6
30	11.8	5.3	6.5	11.8	5.3	6.5	11.8	5.3	6.5	11.1	4.9	6.2	10.9	4.8	6.1	10.7	4.7	6
% Change over control	0	0	0	0	0	0	0	0	0	5.9	7.5	4.8	7.6	9.4	6.1	9.3	11.3	7.6
45	11.8	5.3	6.5	11.8	5.3	6.5	11.8	5.3	6.5	10.7	4.7	6	10.5	4.6	5.9	10.4	4.5	5.9
% Change over control	0	0	0	0	0	0	0	0	0	9.3	11.3	7.6	11.0	13.2	9.2	11.8	15	9.2
60	11.8	5.3	6.5	11.8	5.3	6.5	11.8	5.3	6.5	10.3	4.5	5.8	10.1	4.4	5.7	10.1	4.3	5.8
% Change over control	0	0	0	0	0	0	0	0	0	12.7	15.0	10.7	14.4	16.9	12.3	14.4	18.8	10.7
75	11.8	5.3	6.5	11.8	5.3	6.5	11.8	5.3	6.5	10	4.4	5.6	9.7	4.2	5.5	9.6	4	5.6
% Change over control	0	0	0	0	0	0	0	0	0	15.2	16.9	13.8	17.7	20.7	15.3	18.6	24.5	13.8
90	11.8	5.3	6.5	11.8	5.3	6.5	11.8	5.3	6.5	9.8	4.3	5.5	9.5	4.1	5.4	9.2	3.9	5.3
% Change over control	0	0	0	0	0	0	0	0	0	16.9	18.8	15.3	19.4	22.6	16.9	22.0	26.4	18.4

LSD (P = 0.01) TS – Inc. Period – 0.000708, Relative humidity – 1, 99 E -06  
 RS - Inc. Period – 0.000793, Relative humidity – 1, 09 E -06  
 NRS - Inc. Period – 0.00076, Relative humidity – 5.02 E -08

Table 2. Deterioration of protein ( mg/100mg ) in seeds of *C. corylifolia* under storage at different relative humidity.

Incubation Days	Contro l	33%	Contro l	55%	Contro l	75%	Contro l	96%	Contro l	100%
1	5.88	5.88	5.88	5.88	5.88	5.88	5.88	5.88	5.88	5.88
% Change over control	0	0	0	0	0	0	0	0	0	0
15	5.88	5.88	5.88	5.88	5.88	5.6	5.88	5.4	5.88	5.12
% Change over control	0	0	0	0	0	4.7	0	8.1	0	12.9
30	5.88	5.88	5.88	5.88	5.88	5.04	5.88	4.8	5.88	4.76
% Change over control	0	0	0	0	0	14.2	0	18.3	0	19.0
45	5.88	5.88	5.88	5.88	5.88	4.84	5.88	4.52	5.88	4.4
% Change over control	0	0	0	0	0	17.6	0	23.1	0	25.5
60	5.88	5.88	5.88	5.88	5.88	4.64	5.88	4.32	5.88	4.2
% Change over control	0	0	0	0	0	21.0	0	26.5	0	28.5
75	5.88	5.88	5.88	5.88	5.88	4.44	5.88	4.12	5.88	4.04
% Change over control	0	0	0	0	0	24.4	0	29.9	0	31.2
90	5.88	5.88	5.88	5.88	5.88	4.24	5.88	3.96	5.88	3.88
% Change over control	0	0	0	0	0	27.8	0	32.6	0	34.0

LSD (P = 0.01) – Inc. Period – 0.000738, Relative humidity – 2.34 E -06

Table 3. Deterioration of phenol ( mg/100mg ) in seeds of *C. corylifolia* under storage at different relative humidity.

Incubation Days	Control	33%	Control	55%	Control	75%	Control	96%	Control	100%
1	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9
% Change over control	0	0	0	0	0	0	0	0	0	0
15	2.9	2.9	2.9	2.9	2.9	2.55	2.9	2.5	2.9	2.95
% Change over control	0	0	0	0	0	12.0	0	13.7	0	-1.7
30	2.9	2.9	2.9	2.9	2.9	2.35	2.9	2.4	2.9	2.85
% Change over control	0	0	0	0	0	18.9	0	17.2	0	1.7
45	2.9	2.9	2.9	2.9	2.9	2.2	2.9	2.15	2.9	2.5
% Change over control	0	0	0	0	0	24.1	0	25.8	0	13.7
60	2.9	2.9	2.9	2.9	2.9	2	2.9	1.95	2.9	2.25
% Change over control	0	0	0	0	0	31.0	0	32.7	0	22.4
75	2.9	2.9	2.9	2.9	2.9	1.95	2.9	1.75	2.9	1.9
% Change over control	0	0	0	0	0	32.7	0	39.6	0	34.4
90	2.9	2.9	2.9	2.9	2.9	1.9	2.9	1.7	2.9	1.65
% Change over control	0	0	0	0	0	34.4	0	41.3	0	43.1

LSD (P = 0.01) – Inc. Period – 0.001791, Relative humidity – 1,16 E -05

Table 4. Deterioration of glycoside ( mg/100mg ) in seeds of *C. corylifolia* under storage at different relative humidity.

Incubation Days	Control	33%	Control	55%	Control	75%	Control	96%	Control	100%
1	2.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75
% Change over control	0	0	0	0	0	0	0	0	0	0
15	2.75	2.75	2.75	2.75	2.75	2.69	2.75	2.67	2.75	2.66
% Change over control	0	0	0	0	0	2.1	0	2.9	0	3.2
30	2.75	2.75	2.75	2.75	2.75	2.65	2.75	2.63	2.75	2.61
% Change over control	0	0	0	0	0	3.6	0	4.3	0	5.0
45	2.75	2.75	2.75	2.75	2.75	2.6	2.75	2.57	2.75	2.55
% Change over control	0	0	0	0	0	5.4	0	6.5	0	7.2
60	2.75	2.75	2.75	2.75	2.75	2.56	2.75	2.54	2.75	2.52
% Change over control	0	0	0	0	0	6.9	0	7.6	0	8.3
75	2.75	2.75	2.75	2.75	2.75	2.53	2.75	2.51	2.75	2.49
% Change over control	0	0	0	0	0	8	0	8.7	0	9.4
90	2.75	2.75	2.75	2.75	2.75	2.50	2.75	2.48	2.75	2.45
% Change over control	0	0	0	0	0	9.0	0	9.8	0	10.9

LSD (P = 0.01) – Inc. Period – 0.000673, Relative humidity – 4, 56 E -07

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#### REFERENCES

Al-Juraifani A A 2011 Natural occurrence of fungi and aflatoxins of Cinnamon in the Saudi Arabia, *African J. of Food Sci.*, **5(8)** 460-465.  
Anyanwu Chukwudi U 2010 Fungal contaminants of powdered herbal drugs sold in parts of Enugu State,

Southeast Nigeria, *Plant Product Research J.* **16** 46-50.  
Sareen A, Ahirwar R, Gautam A & Bhadauria R 2010 Fungal contamination of some common medicinal plants of Himachal Pradesh, *Science and Culture.* **76** (3-4) 118-120  
Dutta GR & Roy AK 1987 Mycoflora associated with *Strychnous* seed deterioration of their active principles under storage, *Indian Phytopath.* **40** (4) 520.  
Inman RE 1962 Disease development, disease intensity and Carbohydrate levels in rusted bean plants,

- Phytopath.* **52** 1207.
- International seed testing association 1966 *International rules for seed health testing, Proc. International seed test assoc.* **31** 1.
- Kokate CK, Purohit AP & Gokhale SB 2002 *Pharmacognosy*, Nirali Prakashan 18<sup>th</sup> Ed., Pp. 238.
- Kirtikar KR & Basu BD 1999 *Indian medicinal plants*. 2<sup>nd</sup> Ed. International Books Distributors, Deharadun., I
- Lowry OH, Rosenbrough NJ, Farr AL & Randal RF 1951 Protein measured with the Folin- Ciocalteu's reagent, *J. Biol. Chem.* **193** 265.
- Marx AF, Beck HC, Vanderwaard WF & Flines de J 1966 Microbial deterioration of materials. In :*Microbial biodeterioration'* (ed. Rose AH) Academic press London, 1.
- Maheshwari RK 1987 Seed borne fungi and protein changes in *Lobia (Vinga sinensis Savi)* seeds, *Geobios*, **14(1)** 33.
- Nadkarni KM 1927 *Indian Materia Medica* I, pp. 35.
- Nelson NA 1941 photometric adaptation of the Somogyi Method for determination of Glucose, *J. Biol. Chem.* **153**, 375.
- Roy AK, Chourasia HK & Kumari N 1987 Association of mycoflora with some crude herbal drug of Bhutan. *Indian Bot. Repr.* **6(1)** 48.
- Rose AH 1981 *Microbial biodeterioration*. Economic microbiology. Academic Press London New York, **6** 1.
- Singh M, Singh SS & Sanwal GG 1978 A new colorimetric method for the determination of phenolics, *Indian J. Exp. Biol.* **16(4)** 712.
- Wagner H 1977 Phenolic compounds in plants of pharmaceutical interest. In: *Biochemistry of plant phenolics*. Ed. T Swain, J B Harborne. CF Van sumere. Pp.589.
- Wink SA & Sears GR 1950 Instrumentation studies LVII equilibrium relative humidities above saturated salt solutions at various temperatures, *Tappi*, **33(9)** 96A.